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INTERNATIONAL APPLICATION PUBLISHED

WO 9604391A1

(51) International Patent Classification 6 :
C12N 15/64, 15/67, 15/85, 9/72, 5/10

A1

(43) International Publication Date: 15 February 1996 (15.02.96)

(21) International Application Number: PCT/US95/09576

(22) International Filing Date: 28 July 1995 (28.07.95)

(30) Priority Data:
08/286,740 5 August 1994 (05.08.94) US

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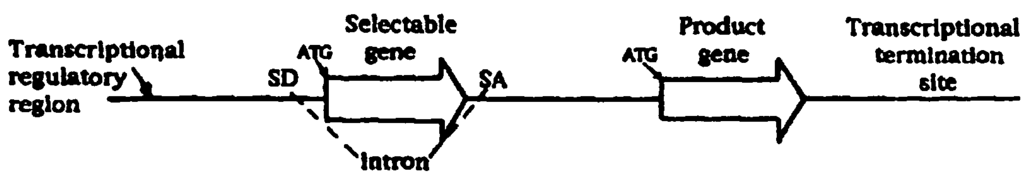
(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS



(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler et *al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., **264(24)**: 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (ΔGT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [ΔGT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ΔGT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	$Na^{+}K^{+}$-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5′ end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3′ end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5′ and 3′ ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5′ end of the intron, where the "exon" comprises the nucleic acid 5′ to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerase chain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Req., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for $DHFR^+$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR[+] cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA 3150

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CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT 3400

TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC 3300

GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT 4350

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

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GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC 2300

ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

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TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA 3100

CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC 3200

CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

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GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG 4650

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ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA 4750

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AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT 5150

CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC 5200

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GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5950

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6000

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6050

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CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6700

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6800

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CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

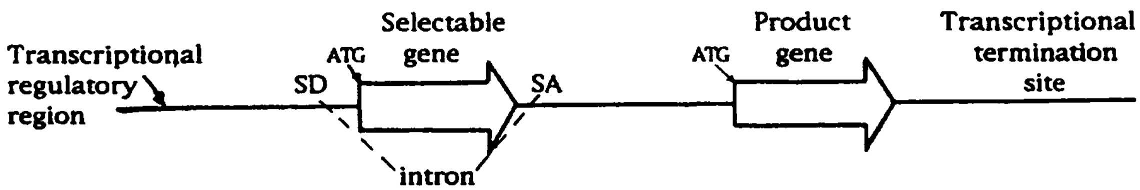
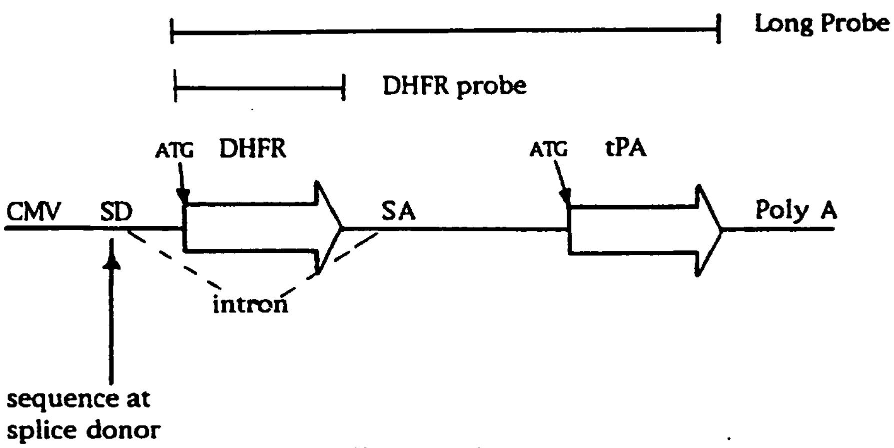
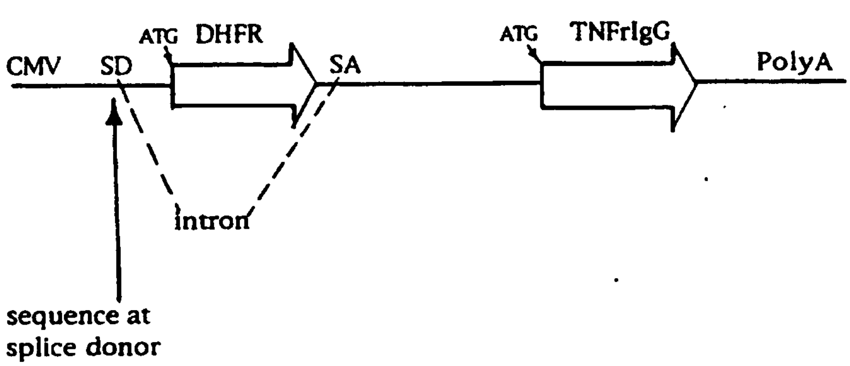


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

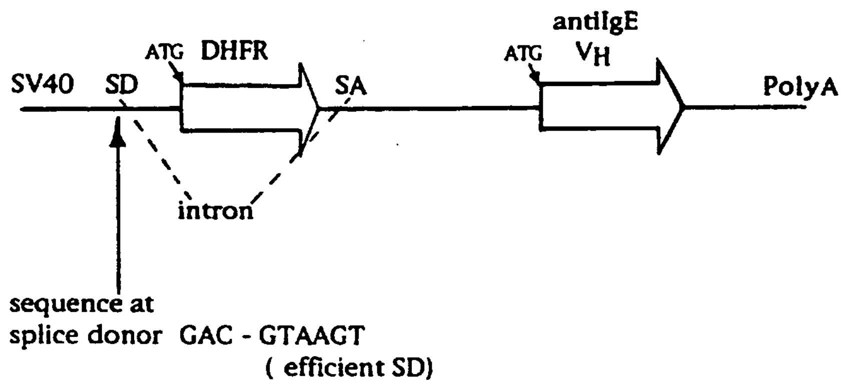

antiIgE
ATG
ATG
DHFR
V_H
SV40
SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

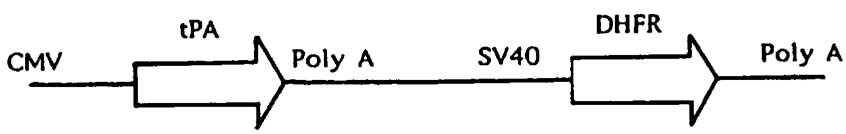

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
       aluI
      sstI
      sacI
      hgiJII
      hgiAI/aspHI
      ecl136II
      bsp1286
      bsiHKAI
      bmyI                     rmaI    tru9I
      banII                    maeI    mseI
    taqI                      speI    aseI/asnI/vspI                    bslI           aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                   scrFI
                   mvaI
                   ecoRII
                   dsaV
                 aciI
               bglI bstNI                                    maeII
              sau96I                                       hinlI/acyI
              haeIII/palI   aciI                           ahaII/bsaHI
              asuI  apyI[dcm+]             aciI     aatII        maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                  maeII
     hinlI/acyI                                                                             hinlI/acyI
     ahaII/bsaHI                                    rsaI                      rsaI          ahaII/bsaHI
     aatII                          bglI            csp6I          ndeI       csp6I         aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

             scrFI
             mvaI
             ecoRII
         aciI                                                                                         nlaIII
       bglI  dsaV                                                                               styI
      sau96I bstNI         rsaI                                            maeII                      ncoI
      haeIII/palI          csp6I                               rsaI       snaBI                       dsaI hphI  aciI
      asuI   apyI[dcm+]  bsrI  nlaIII                          csp6I      bsaAI                       bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                                              maeII
                                                                                            hinlI/acyI                nlaIV
                rsaI                                  pleI                                  ahaII/bsaHI               hgiCI
                csp6I                     aciI        hinfI                    bsmAI        aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                              aluI
                                                                                                             sstI
                                                                                                             sacI
                                                                                                             hgiJII
                                                                                                             hgiAI/aspHI
                                                                                                             ecl136II
                                                                                                             bsp1286
                                                                                                             bsiHKAI
                                                                                     rsaI                    bmyI
                                 maeIII     aciI       hgaI        aciI              csp6I       mnlI         banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                    haeIII/palI
                                                                                                   mcrI
                                                                                                   eagI/xmaIII/eclXI
                                                                                                   eaeI
                                                                                                   cfrI
                                                                                                  fnu4HI
                                                                                                  aciI
                                                                                                 thaI
                                 esp3I                                                           fnuDII/mvnI
                            scrFI                                               sau96I     sacII/sstII
                            mvaI bsmAI                                                avaII      nspBII
                            ecoRII                                              asuI       kspI  scrFI
                            dsaV                                               nlaIV       dsaI  nciI
                            bstNI  hinlI/acyI                                      scrFI     bglI bslI  mspI
                            apyI[dcm+]                                             nciI  sau3AI mnlI bstUI
                       sau3AI gsuI/bpmI                                            mspI  mboI/ndeII[dam-] hpaII
                       mboI/ndeII[dam-]                                     mboII  hpaII  dpnI[dam+] bsaJI dsaV
                       dpnI[dam+]  hgaI  fokI                               bpuAI  dsaV   dpnII[dam-] bsh1236I
                       dpnII[dam-] ahaII/bsaHI              mnlI            bbsI   cauII  alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
          tfiI
        aciI
       thaI hinfI
       fnuDII/mvnI
       bstUI                                                                                      fnu4HI
       bsh1236I                                    aciI                                           bbvI
                                               rsaI                                            nspBII
                                               csp6I    scfI                                   aciI          taqI
                                                                          mnlI                            nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                       thaI
                                                                                       fnuDII/mvnI
                                                                                       bstUI
                                                                                       bsh1236I
                                                                                     mluI
                                                                          bsrBI      aflIII       rsaI
                 pflMI                         bsmAI                      aciI      xmnI          csp6I
     sfaNI       bslI                          bsaI                    mnlI    ddeI asp700       scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
                                                            ecoRII
                                                            dsaV
       eco57I                                               bstNI
      mboII                     tfiI                        apyI[dcm+]              tfiI       tru9I        tru9I
     earI/ksp632I               hinfI                                           mboII  taqI    mseI         mseI
     mnlI                      alwNI     hphI             sexAI           ddeI   hinfI       ahaIII/draI  aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                          aluI
                                         sstI
                                         sacI
                                         hgiJII
                                         hgiAI/aspHI
                                         ecl136II
                                         bsp1286
                                         bsiHKAI
                                         bmyI                                               tru9I
                                         banII                                  sfaNI      mseI           mspI
       ddeI                     bslI   mnlI                    bstXI      fokI             aflII/bfrI     hpaII
                                                                                                        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                                    haeIII/palI
                                                                                  haeI
                                                              scrFI          scrFI                             nlaIII
                                                              mvaI                 mvaI                              sau3AI
                                                              ecoRII         ecoRII                         mboI/ndeII[dam-]
                                                              dsaV   tfiI    dsaV    pleI                   dpnI[dam+]
                                                              bstNI    nlaIII    bstNI     ddeI                    dpnII[dam-]
              accI  nlaIII                    mnlI            apyI[dcm+]  hinfI  apyI[dcm+]   hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                         mnlI
                                                                                    hinlI/acyI          scrFI
                                                                                    ahaII/bsaHI         mvaI
                                                                                 scrFI          ecoRII
                                                                                 mvaI    ecoNI          dsaV
                                                                                 ecoRII     sau96I
                                                                                 dsaV       avaII
                        maeII                                                    bstNI   bslI    asuI    mnlI
                      aflIII                                                     apyI[dcm+]    mnlI  bstNI
          apoI     maeIII                                       mnlI           bsaJI hgaI     ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                 ppu10I
                                                               sfaNI                             nsiI/avaIII
          sfaNI                  accI     mboII             mboII                      mnlI    aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                            fnu4HI
        nlaIII                                              aciI
       styI                                                thaI
       ncoI                                                fnuDII/mvnI tru9I
       dsaI                       styI                     bstUI       mseI                                  maeIII
       bsaJI                      bsaJI                    bsh1236I   aseI/asnI/vspI                        hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

```
                                                     scrFI
                                                     mvaI
                                                     ecoRII                                         bspMI
                                                     dsaV                                         scfI
                                                     bstNI                                              pstI
                                                     apyI[dcm+]       mnlI         aluI     bsgI                  tru9I
                                                   gsuI/bpmI          bsaJI        hindIII  fnu4HI                mseI
       scfI         fokI                        maeIII           hincII/hindII    ddeI      bbvI               apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                          hgiJII                                                          scrFI
                                          bsp1286                                                         mvaI
                                          bmyI                fnu4HI                                      ecoRII
                             sfaNI      mnlI                  bbvI                   mboII                dsaV
                              fokI     mboII  banII        fnu4HI                   bpuAI                 bstNI
         hgaI              nlaIII      earI/ksp632I        bbvI                     bbsI                  apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                                scfI
                             sau3AI             pstI
                             mboI/ndeII[dam-]   bsgI                                                        hgiAI/aspHI
                        nlaIV  dpnI[dam+]     sau3AI                                                 hinPI  bsp1286
                      mnlI   bstYI/xhoII      mboI/ndeII[dam-]                                  fnu4HI      bsiHKAI
        tfiI       mboII     bglII            dpnI[dam+]                                        bbvI        bmyI
        hinfI      earI/ksp632I dpnII[dam-]   dpnII[dam-]                                   nlaIII  hhaI/cfoI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

                   scrFI
                   nciI
                   mspI
                   hpaII                                         alwNI
                   dsaV                              bsp1286    ddeI                  fnu4HI   styI
       ddeI        cauII     sspI                    bmyI      draIII                 bbvI     bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```

FIG. 3F

```
                                                                                                             pmlI
                                                                                                             eco72I
                                                                                                         sau96I
                                                                                                         asuI maeII
                                                                                                     scrFI
           bspMI                                                                                     mvaI haeIII/palI
        nlaIV          sau96I                                                                        ecoRII
        hgiCI          haeIII/palI                                                                   dsaV
        banI           asuI   rsaI                          bsp1286                                  bstNI   bsaAI
       bsp1286        eco0109I/draII                        bmyI                                     bsaJI   bbrPI
       bmyI          alwNI    csp6I ddeI                 bsrI      bcgI                              apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

                scrFI                                     hinPI
                mvaI                                            hhaI/cfoI
                ecoRII                                   nlaIV    hgiAI/aspHI
                dsaV                                     narI     bsp1286                thaI
                bstNI                  pmlI  hgiAI/aspHI       kasI     bsiHKAI                fnuDII/mvnI
                bsaJI                  eco72I                  hinlI/acyI                      bstUI sau96I
            sau96I                bsaAI bsp1286          hgiCI    bmyI                   hinPI  haeIII/palI
            avaII                 bbrPI bsiHKAI          haeII    apaLI/snoI             hhaI/cfoI
            asuI     sfaNI   scfI  bsp1286   bmyI    aciI      banI     alw44I/snoI       fnu4HI     asuI        bslI
          mnlI  apyI[dcm+] aluI    bmyI maeII      nspBII      ahaII/bsaHI    bsrI        bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                            scrFI
                                            mvaI
                                            ecoRII
                                            dsaV                                  pleI      scrFI
                                            bstNI                                     bsmAI      mvaI
                                            bsaJI                                  taqI[dam-]    ecoRII
                         hinlI/acyI         apyI[dcm+]                            sau3AI hinfI   dsaV
              aciI  haeIII/palI           haeIII/palI           scfI              mboI/ndeII[dam-] bstNI
        nspBII     haeI  hgaI bstXI      sau96I           pstI          dpnI[dam+]        apyI[dcm+]      tru9I
     scfI aciI    mnlI   ahaII/bsaHI     asuI      nlaIV        bsgI          dpnII[dam-]      bsaJI      maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

```
                                   fnu4HI
                                   bbvI
                     aluI        scfI
                 rsaI            pstI                    mnlI       maeIII                               dsaI
        aciI     csp6I ddeI      bsgI                  ddeI       alwNI                                  bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
     TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
                                                                                                    scrFI
                                                                                                    pflMI
                                                                                                    mvaI
                                                                                                    ecoRII
                                                                                                    dsaV
                                                       sau3AI                                       bstNI
                                                       mboI/ndeII[dam-]                             bslI
                                                       dpnI[dam+]                                   apyI[dcm+] haeIII/palI
                 pleI  nlaIV               ecoRI       dpnII[dam-]                              bsp1286       sau96I
           hphI  hinfI hgiCI          dsaI apoI        alwI[dam-]                               bmyI alwNI    asuI
         mnlI   bcgI   banI mnlI    mnlI bsaJI      nlaIII                                   bsrI  bsaJI    bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
     TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
                                                            nlaIV
                                                            hgiCI
                                                            banI  maeII
                                                        scrFI
     scrFI                                         mvaI    pmlI
     mvaI                                                ecoRII
     ecoRII                                        dsaV
     dsaV                  tfiI                    bstNI    eco72I
     bstNI                       hinfI             pflMI    bsaAI
     bsaJI                     mspI            sfaNI    apyI[dcm+]       mboII                    rsaI
     apyI[dcm+]                hpaII          fokI  bslI bsaJI     bbrPI  eco57I  aciI     maeII  csp6I     bsp1286
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
     GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
                                                                                                    scrFI
                                                                           mboII                    mvaI
                                                                           earI/ksp632I             ecoRII
                                                                        hgiJII                      dsaV
                                                                        bsp1286                     bstNI
                  fnu4HI  bsmAI                                         bmyI                        apyI[dcm+]
                  aciI  ddeI    rsaI        ddeI                        banII                  bslI bsaJI    fnu4HI
       mnlI     bspMI haeIII/palI csp6I    mnlI        sfaNI        mnlI sapI              mnlI    bslI      bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
     GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC
```

FIG. 3H

```
                              mspI
                              hpaII
                              bslI
                             scrFI                                                                     scrFI
                             nciI       nlaIV                                                          mvaI
                             dsaV     aciI                              aluI        tfiI       aciI    ecoRII
                                                                                                       dsaV
                                                                                                       bstNI
          bslI       mnlI    cauII  bsrBI     bslI aciI                 alwNI       hinfI      fnu4HI  apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                             scrFI
                                                             mvaI
                                                             ecoRII
                                                             dsaV
                                                             bstNI
                                                             apyI[dcm+]
                                                    scrFI    bslI
                                   sau3AI           nciI     bsaJI
                                   mboI/ndeII[dam-) mspI   sau96I
                                   dpnI[dam+]       hpaII  nlaIV
                                   dpnII[dam-]      dsaV   avaII          mnlI
   mnlI     aciI     bslI        hphI[dam-]         cauII  asuI       mnlI           apoI        taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                    scfI                                 scrFI
                                                    pstI                                 mvaI
                                                   fnu4HI                                ecoRII
                                                   bbvI pvuII                            dsaV
                                                fnu4HI fnu4HI                            bstNI         aciI
                                                bbvI   bbvI                         bsp1286      fnu4HI sau96I
        ecoRI                                 hinPI bsgI aluI     tfiI      nspBII  bsaJI        bbvI  avaII
        apoI taqI                             hhaI/cfoI nspBII    hinfI     aciI bmyI apyI[dcm+]       asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                          aluI
                                                          sstI
                                 pvuII                    sacI
                     mspI     scfI                        hgiJII
                     hpaII    pstI  fnu4HI                hgiAI/aspHI
                    scrFI    bspMI  bbvI                  ecl136II                  haeIII/palI
                    nciI   sau96I nspBII                  bsp1286                 stuI
                    dsaV   avaII fnu4HI    bsrI           bsiHKAI                 haeI
                    cauII  asuI bbvI    mspI              bmyI   mspI            mnlI
      draIII   bslI bsaJI aciI bsgI aluI hpaII            banII  hpaII        nlaIII     bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                fnu4HI
                                              nspBII
        fnu4HI                                aciI                                         nlaIII
        aciI                       rsaI fokI  fnu4HI                 tru9I       hphI     nspI
      bsrBI eco57I mnlI nlaIII     csp6I   alwNI bbvI                mseI       maeIII    nspHI       gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                       sau96I
                       nlaIV
                       haeIII/palI
                       asuI
                      sau96I
                      nlaIV scrFI                                  scrFI
                      hgiJII                                            mvaI
                      eco0109I/draII               scrFI           ecoRII
                      bsp1286                      mvaI                 dsaV
                      bsp120I                      ecoRII          bstNI
                      bmyI  mvaI                   dsaV       sau96I
                      banII ecoRII            bstNI           nlaIV                    aciI
                    aciI    dsaV              bsaJI           haeIII/palI             fnu4HI
                  fnu4HI    bstNI          hinlI/acyI     tfiI    asuI apyI[dcm+]          haeIII/palI
                  aciI asuI apyI[dcm+]     hgaI    apyI[dcm+]      eco0109I/draII         eaeI
      bsmAI     bsrBI apaI bsaJI           ahaII/bsaHI    hinfI  mnlI bsaJI              cfrI  nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        eco0109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII               tfiI        nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI             rmaI     hinfI       nspHI
           sfaNI   nspBII  haeIII/palI            fokI avaI     bsp1407I   bstEII        maeI bsrI      maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                             mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                       mboI/ndeII[dam-]
                dsaV                         dpnI[dam+]                 mboII
                bstNI            mnlI              dpnII[dam-]          eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]          mboII  bpuAI           hinPI
      mcrI maeIII            hinfI                bstYI/xhoII   mboII      bbsI               hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI               mboII
      gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI earI/ksp632I                                tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                    scrFI
                                                                  scrFI       mvaI
                                                                  mvaI bsmI         ecoRII
                                                                  ecoRII      dsaV
                                                                  dsaV        bstNI
                                      mboII                       bstNI             bsaJI
                                      bpuAI   bsmI      rmaI    gsuI/bpmI   mnlI   bsaJI                 ecoRI   taqI
                                      bbsI nlaIII       maeI mnlI apyI[dcm+]   mnlI apyI[dcm+]           apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
         styI
      aciI
     fnu4HI   sau96I
     bglI nlaIII
    sfiI ncoI haeIII/palI
    haeIII/palI                alwI
   eaeI  dsaI asuI          fnu4HI
   cfrI  bsaJI              bbvI          maeIII              sfaNI  apoI                     bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                         sau3AI
                                                         mboI/ndeII[dam-]
                                                         dpnI[dam+]
                                                         dpnII[dam-]
                                                        pvuI/bspCI
                                                        mcrI
                                                       taqI[dam-]  tru9I
                                                     claI/bsp106[dam-]          styI
                                                    sau3AI        mseI      fnu4HI    haeI
                                                    mboI/ndeII[dam-]        bbvI   ncoI
                                                    dpnI[dam+] xmnI       hinPI    dsaI haeIII/palI
   rmaI                                             dpnII[dam-]   aseI/asnI/vspI   bsaJI
   maeI                                         nlaIII alwI[dam-]   asp700    hhaI/cfoI nlaIII       mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                  rsaI                                                                 nlaIV
                  csp6I                                                             scrFI
                 nlaIV                                                              mvaI
                 kpnI                                                               ecoRII
                 hgiCI                                                              dsaV
                 banI                       aluI                                    bstNI
                 asp718     mnlI           pvuII                                    apyI[dcm+]
   mnlI          acc65I   ddeI aciI        nspBII                                  bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                         nlaIV
           sfaNI                     scrFI        scrFI                                    sfaNI
         ppu10I                      mvaI               mvaI                                         ppu10I
         nsiI/avaIII                 ecoRII       ecoRII                                 nsiI/avaIII
        nlaIII                       dsaV         dsaV                                  nlaIII
       sphI                          bstNI              bstNI                                      sphI
       nspI                          apyI[dcm+]         apyI[dcm+]                                 nspI
       nspHI                        sexAI              bsaJI                                       nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                        nlaIII
                                                                                       styI
                                                                                       ncoI
                                        aciI                                      bslI dsaI
           aciI         aciI   fokI                 aciI bsrI  aciI               aciI bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                              rmaI
                                                                             styI
            aciI                                                             bsaJI
           fnu4HI                                                            blnI
          sfiI                                                               avrII
          haeIII/palI                                                      haeIII/palI           tru9I
      bsaJI bglI         ddeI                                             stuI                 hpaI
    haeIII/palI mnlI haeIII/palI                        mnlI              haeI                 hincII/hindII
  mnlI    mnlI   bsaJI   mnlI   aluI                mnlI              mnlI maeI            aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                            scrFI
                                            mvaI
                                            ecoRII
   haeIII/palI                              dsaV
  eaeI                                      bstNI                                                 aluI
  cfrI                       bsrI           apyI[dcm+]      tru9I        fnu4HI                  pvuII
 bsrI                  maeII maeIII        bsaJI    maeIII   mseI         bbvI    fokI           nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

```
                                                                      hinPI
                                                                      hhaI/cfoI
                                                                     nlaIV
                 sau3AI                                              narI
       sau96I    mboI/ndeII[dam-]                                    kasI
       haeIII/palI                                                   hinlI/acyI
       asuI      dpnI[dam+]                                          hgiCI
     mnlI        dpnII[dam-]                                         haeII     aciI
  mboII   aciI  pvuI/bspCI                                           banI    sfaNI
  earI/ksp632I  mcrI                           bglI                  ahaII/bsaHI                    sfaNI    aciI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
     GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA

                                                                       aciI
                                                                    fnu4HI                   fnu4HI
                                        hinPI                      thaI                     hinPI
                                       thaI                        fnuDII/mvnI              hhaI/cfoI
                                       fnuDII/mvnI                 bstUI                   thaI
                                       bstUI scfI     hinPI     hinPI                      fnuDII/mvnI
                                       bsh1236I       hhaI/cfoI hhaI/cfoI                  bstUI
                                   rsaI hhaI/cfoI  fnu4HI    tru9I aciI                    bsh1236I    aciI
       aciI   maeII                csp6I   bslI    aciI      mseI bsh1236I              maeIII bbvI  maeIII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
     AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG

             hinPI
             hhaI/cfoI                                                                         nlaIV
         rmaI                                                       mspI                      hgiJII
   hinPI  haeII                                                     hpaII                     bsp1286
   hhaI/cfoI   bsrBI                                               naeI                       bmyI
  haeII maeI   aciI                  mboII                  maeII  cfr10I          aluI      banII        nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
     GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC

                   nlaIV                                              maeII    haeIII/palI
                   hgiCI  taqI                                       draIII   sau96I
                   banI mnlI                             hphI        bsaAI    asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
     AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA

   maeII pleI          tru9I      pleI                          bslI
  drdI   hinfI  maeII  mseI       hinfI        bsrI            bslI  avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
     ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA
```

FIG. 3N

```
                                                                                                       hgiAI/aspHI
                                                            thaI                                       bsp1286
                                                            fnuDII/mvnI             maeII              bsiHKAI
                        tru9I                          tru9I   apoI tru9I          psp1406I            bmyI    ddeI
                        mseI              tru9I        mseI bstUI   mseI         tru9I                 apaLI/snoI
             haeIII/palI          aluI    mseI    apoI      bsh1236I        sspI mseI                  alw44I/snoI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
     CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG

                                                                                                               hinPI
                                                                                                               hhaI/cfoI
                                                                                                              thaI
                                                                              hinPI                           fnuDII/mvnI
                        aciI                                    maeIII      fnu4HI                            bstUI
     rsaI              fnu4HI    tru9I                       maeII bsrI  nlaIII hhaI/cfoI                  nspBII bsh1236I
     csp6I          sfaNI        mseI        aciI           bsaAI tth111I/aspI bbvI               aciI     aciI hgaI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
     TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG

                                                                 scrFI
                               sfaNI                             nciI
                            mspI                                 mspI
                            hpaII                                hpaII    nspI
                           scrFI                                 dsaV     nspHI
                           nciI                              esp3I     fnu4HI
                           dsaV fokI                   maeIII bsmAI    bbvI
         drdI              cauII   aciI          aluI     bslI  cauII aluI nlaIII   mnlI    hphI     hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
     CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT

      thaI
      fnuDII/mvnI
      bstUI
      bsh1236I
     hinPI
     hhaI/cfoI                          mnlI
     thaI mnlI                        haeIII/palI                                                 hinlI/acyI
     fnuDII/mvnI        mboII       sau96I                               nlaIII                   ahaII/bsaHI
     bstUI              bpuAI       asuI                           tru9I rcaI                     aatII
     bsh1236I           bbsI       eco0109I/draII                  mseI  bspHI                ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
     GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT
```

FIG. 3O

```
                        nlaIV
                      aciI
                     thaI
                     fnuDII/mvnI
                     bstUI
                     bsh1236I
                    hinPI                                                                  rcaI
                    hhaI/cfoI                                                              bspHI
                                                                                       bsrBI    bsmAI
                                                                                       aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                  mboII                                                               fnu4HI
     sspI         earI/ksp632I                                                        aciI                         hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                         hgiAI/aspHI
                                         bsp1286
                               sau3AI    bsiHKAI                         sau3AI   nspBII    sau3AI
                               mboI/ndeII[dam-]                          mboI/ndeII[dam-]   mboI/ndeII[dam-]
                               dpnI[dam+] bmyI                           dpnI[dam+]         dpnI[dam+]
                               dpnII[dam-]                               bstYI/xhoII        dpnII[dam-]
                          eco57I         apaLI/snoI                 bsrI dpnII[dam-]        alwI[dam-]
       hphI          sfaNI  mboII[dam-]  alw44I/snoI  maeIII taqI   alwI[dam-]   aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                                scrFI
                                                     aciI                       nciI
                                                   thaI                         mspI
        maeII                                      fnuDII/mvnI                  hpaII
       psp1406I                 hgiAI/aspHI        bstUI                        dsaV
       xmnI                     bsp1286  tru9I     bsh1236I                 hinlI/acyI
       asp700                   bsiHKAI  mseI     hinPI                     hgaI cauII                     aciI
     mboII                      bmyI   ahaIII/draI hhaI/cfoI                ahaII/bsaHI    bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                               rsaI
                               csp6I   bsrI                                                           fnu4HI
          ddeI                scaI  hphI  maeIII    sfaNI    fokI   nlaIII                            bbvI       nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

```
                                                      sau96I
                                                      avaII
                                              sau3AI  asuI                                          nlaIII
                       haeIII/palI            mboI/ndeII[dam-]                                   sau3AI maeIII
                      eaeI                    dpnI[dam+]                                         mboI/ndeII[dam-]
                     cfrI                     dpnII[dam-]                                        dpnI[dam+]
                     fnu4HI                  pvuI/bspCI                                          dpnII[dam-]
                     aciI                    mcrI   mnlI         aluI   aciI             nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                hinPI
                    mspI                                                                        hhaI/cfoI
       sau3AI   nlaIV                                                                          mstI
       mboI/ndeII[dam-] aluI                                                                   aviII/fspI      bsrI
       dpnI[dam+]   hpaII                                                       fnu4HI         maeII       tru9I
       dpnII[dam-] bsaWI                                     maeIII     sfaNI   bbvI        psp1406I       mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                               mspI
                               hpaII                                                              bglI
                              scrFI                                                              sau96I
                        aluI  nciI        tru9I      fokI                       sau96I           haeIII/palI
                      rmaI    dsaV        mseI    bsrI        aciI              avaII       hinPI asuI      mspI
                      maeI    cauII      aseI/asnI/vspI  mnlI                   asuI        hhaI/cfoI       hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATG GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                                mspI                thaI
                                hpaII               fnuDII/mvnI             haeIII/palI
                               cfr10I               bstUI                  sau96I
                            nlaIV hphI         bsmAI aciI          fnu4HI  nlaIV
                         gsuI/bpmI            bsaI  bsh1236I       bbvI bsrI asuI                mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                         ddeI
                                                    sau3AI        nlaIV
                pleI                                mboI/ndeII[dam-]
                hinfI                               dpnI[dam+]    hgiCI         tru9I
         eam1105I               fokI                dpnII[dam-]   banI mnlI     mseI          maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

FIG. 3Q

```
                                                             rmaI      sau3AI
                                                         sau3AI hphI   mboI/ndeII[dam-]
                                                         mboI/ndeII[dam-]
                                                         dpnI[dam+]    dpnI[dam+]
                                                         dpnII[dam-]   dpnII[dam-]
                             tru9I                tru9I bstYI/xhoII    alwI[dam-]        nlaIII              maeII
                             mseI          tru9I mseI  alwI[dam-]   bstYI/xhoII         rcaI              tru9I
                            ahaIII/draI    mseI ahaIII/draI maeI  mboII[dam-]           bspHI             mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                       sau3AI
                                                       mboI/ndeII[dam-]
                                                       dpnI[dam+]  sau3AI
                                                       dpnII[dam-] mboI/ndeII[dam-] thaI
                                                      bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                              sau3AI  alwI[dam-]   dpnII[dam-]      bstUI
                                              mboI/ndeII[dam-]     alwI[dam-]       bsh1236I
                        hgaI                  dpnI[dam+] mboII[dam-]               hinPI       fnu4HI
                    ddeI                      dpnII[dam-]         bstYI/xhoII      hhaI/cfoI   bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                         sau3AI
                                         mboI/ndeII[dam-]
                                         dpnI[dam+]
                                         dpnII[dam-]
                                        alwI[dam-]
                    aciI            mspI                                bsrI             hinPI
          aciI    nspBII            hpaII     aluI                   maeIII   eco57I     hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                      fnu4HI
                                                                                               alwNI  bbvI
         rmaI                haeIII/palI                                                      bsrI fnu4HI
         maeI      bslI     haeI                  scfI   aciI        mnlI               maeIII    bbvI     bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

```
                scrFI                                         aciI                               hgiAI/aspHI
                nciI                                         nspBII                              bsp1286
                mspI                            mspI        fnu4HI                               bsiHKAI
                hpaII                           hpaII       bbvI                                 bmyI
                dsaV      pleI                 bsaWI      hinPI  mcrI                            apaLI/snoI
                cauII     hinfI               maeIII      hhaI/cfoI                              alw44I/snoI   aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
     CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA

                                                                                                    mspI
                                                            hinPI                                   hpaII
                                                            hhaI/cfoI                               bslI    fnu4HI
                      ddeI       scfI                      haeII                          aciI     bsaWI   aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
     ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

                                        scrFI
                                        mvaI           scrFI
                                        ecoRII   mvaI
                                        dsaV     ecoRII
                                        bstNI          dsaV
                   hinPI mnlI           bsaJI          bstNI                                              taqI
                   hhaI/cfoI     aluI   apyI[dcm+]     apyI[dcm+]                          mnlI drdI    hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
     GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA

                                                                                haeIII/palI
                                                            haeIII/palI       scrFI
                                                           fnu4HI             mvaI bslI
                                                           aciI               ecoRII
                                                          thaI bslI           dsaV                    nlaIII
                                                          fnuDII/mvnI         bstNI                      nspI
                                   nlaIV                  bstUI               apyI[dcm+]    haeIII/palI  nspHI
              sfaNI              aciI                     bsh1236I      nlaIV  haeI          haeI        aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
     GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT

                                                                                 fnu4HI           fnu4HI
                                                                                 bbvI             bbvI pleI
                         tfiI                                             bsrBI  aciI            hinPI  hinfI
                         hinfI                 aciI          aluI         aciI  fnu4HI     mcrI  hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
     ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT
```

FIG. 3S

```
                                                                                      thaI
                                                                                      fnuDII/mvnI
                                                                                      bstUI
                                                                                      bsh1236I
                                                                                     hinPI
                                                                                     hhaI/cfoI
                                                                                    thaI
                                                                                    fnuDII/mvnI
                             sapI  hinPI                                            bstUI   haeIII/palI        aluI
                             mboII hhaI/cfoI                                        bsh1236I           tru9I  pvuII
                             earI/ksp632I                      mnlI                 bslI    eaeI   tfiI aseI/asnI/vspI
                   mnlI   aciI    haeII                     aciI                    aciI    cfrI   hinfI mseI    nspBII             bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                                          scrFI
                                                                                          mvaI
                                                                                          ecoRII
                                                                                          dsaV
                                               tru9I                                nlaIV bstNI
                                  hinPI        mseI       maeIII                    hgiCI apyI[dcm+]                mspI
              aciI                hhaI/cfoI   aseI/asnI/vspI  mnlI                   banI bsaJI                     hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                                     tru9I
                                                                                     mseI
                                                                                    aseI/asnI/vspI
                 aciI                                                               xmnI
               bsrBI                               aluI        nlaIII               asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT

>length: 7360
```

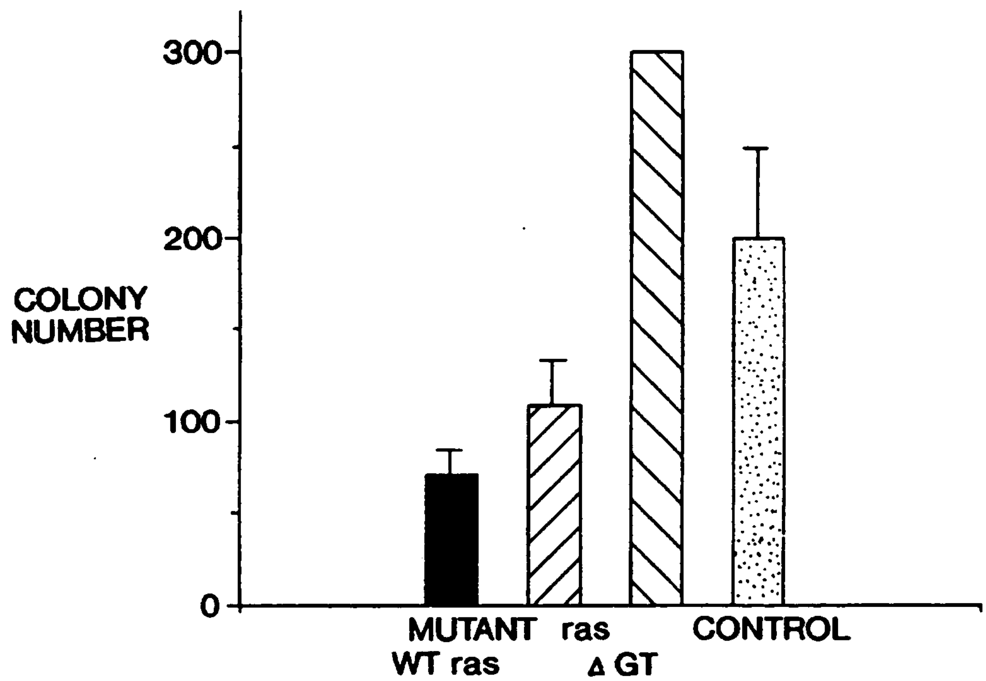

300
200
100
0
COLONY
NUMBER
MUTANT
WT ras
ras
Δ GT
CONTROL

FIG. 4

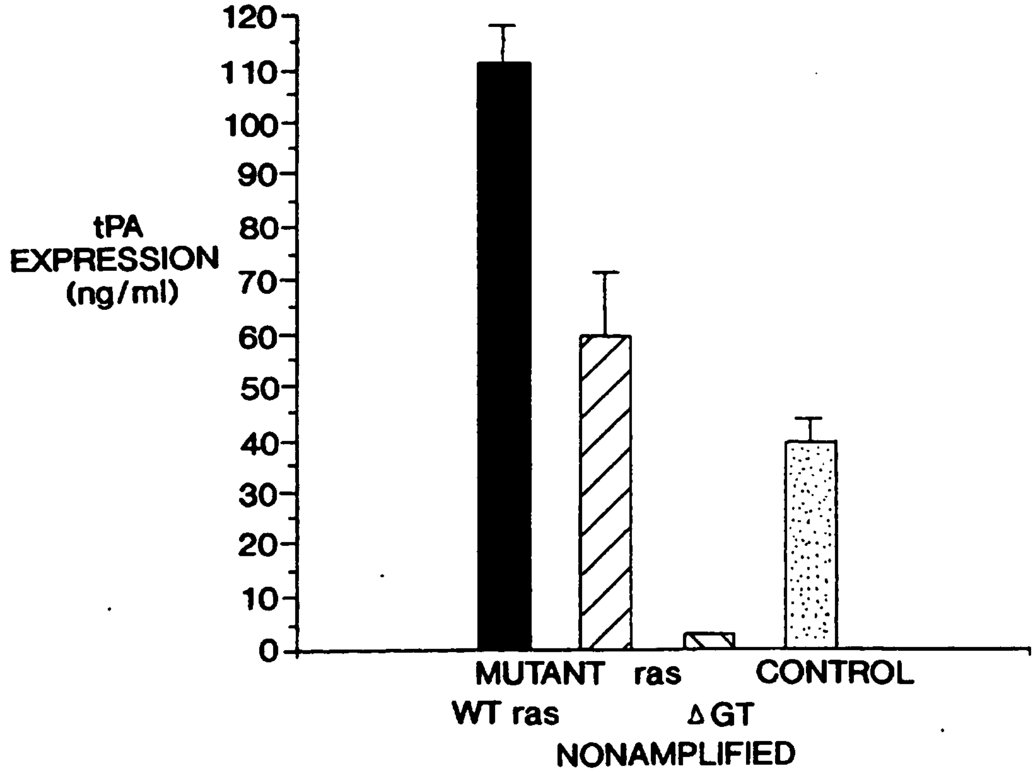

120
110
100
90
80
70
60
50
40
30
20
10
0
tPA
EXPRESSION
(ng/ml)
MUTANT
WT ras
ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

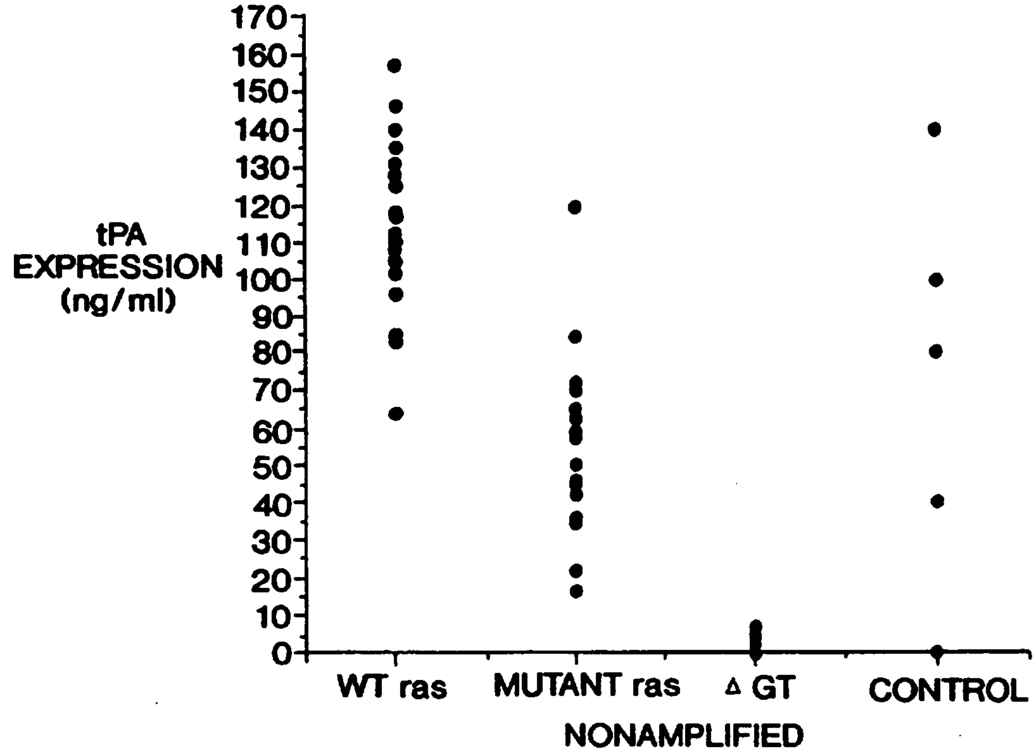

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

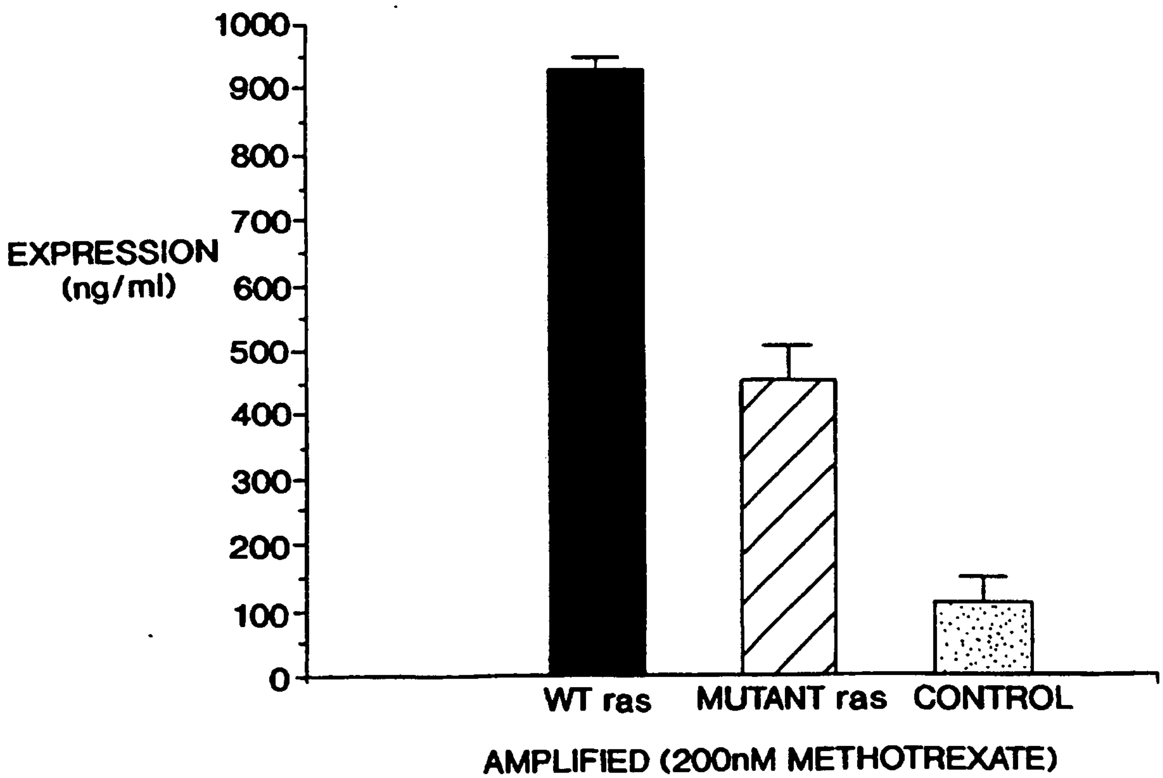

1000
900
800
700
600
500
400
300
200
100
0
EXPRESSION (ng/ml)
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
     aluI
    sstI
    sacI
    hgiJII
    hgiAI/aspHI
    ecl136II
    bsp1286
    bsiHKAI
    bmyI                                                                                                   thaI
    banII                          rmaI    tru9I                                                        fnuDII/mvnI
   taqI                            maeI    mseI                                                         bstUI
                                  speI   aseI/asnI/vspI                              bslI              bsh1236I
                                                                                                       aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
              aciI
             bglI bstNI                                 maeII
            sau96I                                    hinlI/acyI
            haeIII/palI    aciI                       ahaII/bsaHI
            asuI  apyI[dcm+]              aciI        aatII        maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

     maeII                                                                                            maeII
    hinlI/acyI                                                                                      hinlI/acyI
    ahaII/bsaHI                                    rsaI                        rsaI                 ahaII/bsaHI
    aatII                            bglI          csp6I          ndeI         csp6I                aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
       aciI                                                                                            nlaIII
      bglI  dsaV                                                                                 styI
     sau96I bstNI          rsaI                                                  maeII                 ncoI
     haeIII/palI           csp6I                                       rsaI     snaBI                  dsaI hphI  aciI
     asuI   apyI[dcm+]   bsrI  nlaIII                                  csp6I    bsaAI                  bsaJI     sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

```
                                                                            maeII
                                                                            hinlI/acyI            nlaIV
          rsaI                       pleI                                   ahaII/bsaHI           hgiCI
          csp6I              aciI    hinfI                bsmAI             aatII                 banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                   aluI
                                                                                                  sstI
                                                                                                  sacI
                                                                                                  hgiJII
                                                                                                  hgiAI/aspHI
                                                                                                  ecl136II
                                                                                                  bsp1286
                                                                                                  bsiHKAI
                                                                        rsaI                      bmyI
                          maeIII     aciI       hgaI        aciI        csp6I      mnlI           banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                             haeIII/palI
                                                                                            mcrI
                                                                                            eagI/xmaIII/eclXI
                                                                                            eaeI
                                                                                            cfrI
                                                                                           fnu4HI
                                                                                           aciI
                                                                                          thaI
              esp3I                                                                       fnuDII/mvnI
          scrFI                                                       sau96I        sacII/sstII
          mvaI bsmAI                                                       avaII         nspBII
          ecoRII                                                      asuI          kspI  scrFI
          dsaV                                                       nlaIV          dsaI  nciI
          bstNI  hinlI/acyI                                              scrFI        bglI bslI  mspI
          apyI[dcm+]                                                     nciI     sau3AI mnlI bstUI
     sau3AI gsuI/bpmI                                                    mspI     mboI/ndeII[dam-] hpaII
     mboI/ndeII[dam-]                                             mboII  hpaII    dpnI[dam+] bsaJI dsaV
     dpnI[dam+]  hgaI  fokI                                       bpuAI  dsaV     dpnII[dam-] bsh1236I
     dpnII[dam-] ahaII/bsaHI                           mnlI       bbsI   cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 6C

```
               tfiI
           aciI
          thaI hinfI                                                               fnu4HI
          fnuDII/mvnI                         aciI                                 bbvI
          bstUI                            rsaI                                  nspBII           taqI
          bsh1236I                         csp6I   scfI           mnlI           aciI       nlaIII
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                 thaI
                                                                                 fnuDII/mvnI
                                                                                 bstUI
                                                                                 bsh1236I
                                                                               mluI
                                                                    bsrBI      aflIII     rsaI
                     pflMI                        bsmAI             aciI     xmnI         csp6I
    sfaNI            bslI                         bsaI           mnlI   ddeI asp700      scaI
801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
    CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                           scrFI
                                                           mvaI
                                                           ecoRII
         eco57I                                            dsaV
        mboII                 tfiI                         bstNI                   tfiI       tru9I         tru9I
       earI/ksp632I           hinfI                        apyI[dcm+]          mboII  taqI    mseI          mseI
      mnlI                  alwNI    hphI                sexAI            ddeI    hinfI     ahaIII/draI   aseI/asnI/vspI
901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
    TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                  tru9I              mspI
                                             banII                           sfaNI  mseI               hpaII
           ddeI                   bslI    mnlI                bstXI      fokI      aflII/bfrI        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                              haeIII/palI
                                                                             haeI
                                                      scrFI          scrFI                       nlaIII
                                                      mvaI                 mvaI                           sau3AI
                                                      ecoRII         ecoRII                   mboI/ndeII[dam-]
                                                      dsaV   tfiI    dsaV  pleI               dpnI[dam+]
                                                      bstNI    nlaIII      bstNI     ddeI                 dpnII[dam-]
            accI  nlaIII            mnlI              apyI[dcm+]   hinfI   apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                        mnlI
                                                                                   hinlI/acyI        scrFI
                                                                                   ahaII/bsaHI       mvaI
                                                                                scrFI          ecoRII
                                                                                mvaI    ecoNI        dsaV
                                                                                ecoRII      sau96I
                                                                                dsaV        avaII
                                                                                bstNI   bslI     asuI    mnlI
                        maeII                                                   apyI[dcm+]     mnlI  bstNI
                      aflIII
        apoI       maeIII                                       mnlI           bsaJI hgaI    ddeI    apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                             ppu10I
                                                            sfaNI                            nsiI/avaIII
        sfaNI                 accI     mboII           mboII                           mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                        fnu4HI
                                                        aciI
       nlaIII                                          thaI
      styI                                             fnuDII/mvnI tru9I
      ncoI
      dsaI                           styI              bstUI      mseI                                   maeIII
      bsaJI                          bsaJI             bsh1236I   aseI/asnI/vspI                        hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                                   haeIII/palI
                                                                                                  eaeI
                                                                                            cfrI
                                                                                                 mspI
                                                                                           hpaII
                                                                                          scrFI
                                                                                                nciI
                                                       scrFI
                                                       mvaI
                                                       ecoRII
                                                       dsaV
                                                       bstNI
                                                       apyI[dcm+]      mnlI                  ecoRI  dsaV
                                                     gsuI/bpmI         bsaJI           taqI  apoI   cauII
       scfI        fokI                          maeIII     hincII/hindII             claI/bsp106  bsaJI      aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                         gsuI/bpmI
                                                        scrFI
                                                        mvaI
                                                        ecoRII                                                 mnlI
                                                        dsaV                                                   ecoNI
              mnlI                              nspBII  bstNI                         ddeI                       sau96I
            haeIII/palI                     fnu4HI     hgiAI/aspHI                   mnlI                        nlaIV
           sau96I                        fnu4HI aciI   bsp1286                       eco81I                      avaII
           asuI                          bbvI  fnu4HI  bsiHKAI    aluI               bsu36I/mstII/sauI           asuI  hphI
        nlaIII                      bspMI  bbvI        bmyI apyI[dcm+]             bslI                    bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

        rmaI
        maeI
       styI
       bsaJI
       blnI            mboII                  styI                                         rsaI
       avrII           earI/ksp632I           bsaJI               mnlI         taqI        csp6I             nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                           scrFI
                           nciI
                           mspI
                           hpaII
                           dsaV
                           cauII
                          xmaI/pspAI
                          smaI
                          scrFI
                          nciI
                          dsaV
                          cauII
                          bslI
                        sau96I
                        haeIII/palI
                        asuI
                     scrFI
                     mvaI bsaJI
                     ecoRII
                     dsaV                         nlaIV
       rsaI .        bstNI bsaJI      scfI          fnu4HI                   alwNI
       csp6I         bslI avaI        pstI          aciI        aciI       ddeI
      bsp1407I       apyI[dcm+]       bsgI        bsrBI     hphI   eco57I  mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                   scrFI
                                             mboII                 nciI
                                            earI/ksp632I           mspI
      fnu4HI                              sau3AI                   hpaII
       aluI                               mboI/ndeII[dam-]         dsaV          scfI         mspI
      pvuII                               dpnI[dam+]               cauII         pstI         hpaII
      nspBII                              dpnII[dam-]            sau96I          bsgI       rsaI
     ddeI                                bstYI/xhoII             avaII          fnu4HI      csp6I
     mnlI bbvI                           bglII                   asuI    draIII bbvI  mboII bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

```
                                                                     hgiAI/aspHI                                hgiAI/aspHI
                                                                     bsp1286        scrFI           bsp1286
                                                                     bsiHKAI        mvaI                  bsiHKAI
                                            mnlI               sau96I               ecoRII          bmyI
                                         alwNI                 avaII bmyI mnlI      dsaV            apaLI/snoI
                                         fnu4HI                asuI  apaLI/snoI     bstNI                 alw44I/snoI
                            bsrI      munI bbvI       mnlI   nlaIV  alw44I/snoI    apyI[dcm+]      draIII
2001 CGGCATTATT GGAGTGAAAA CCTTTTCCAG TGCTTCAATT GCAGCCTCTG CCTCAATGGG ACCGTGCACC TCTCCTGCCA GGAGAAACAG AACACCGTGT
     GCCGTAATAA CCTCACTTTT GGAAAAGGTC ACGAAGTTAA CGTCGGAGAC GGAGTTACCC TGGCACGTGG AGAGGACGGT CCTCTTTGTC TTGTGGCACA

                                                                                hgiAI/aspHI
                                                                                bsp1286
                                                                                bsiHKAI
                                                                                bmyI
                                                                          gsuI/bpmI
                                                                         scrFI
                                                                         mvaI   apaLI/snoI
                                                                         ecoRII
                                                                         dsaV
            bspMI                                         scfI           bstNI  alw44I/snoI
       bspMI nlaIII         ddeI              bsmAI      maeIII          apyI[dcm+]
2101 GCACCTGCCA TGCAGGTTTC TTTCTAAGAG AAAACGAGTG TGTCTCCTGT AGTAACTGTA AGAAAAGCCT GGAGTGCACG AAGTTGTGCC TACCCCAGAT
     CGTGGACGGT ACGTCCAAAG AAAGATTCTC TTTTGCTCAC ACAGAGGACA TCATTGACAT TCTTTTCGGA CCTCACGTGC TTCAACACGG ATGGGGTCTA

                                                         aluI
                                                       sstI
                                                       sacI
                                                       hgiJII
                                                       hgiAI/aspHI
                                                       ecl136II                                       bsp1286
                                    nlaIV              bsp1286                                        nlaIV
              bsp1286     pleI   hgiCI                 bsiHKAI              maeIII        nlaIII      hgiCI
          tru9I       mnlI  ddeI                       bmyI                 hphI        nspI  dsaI banI
          mseI bmyI ddeI  hinfI banI                   banII       draIII               nspHI bsaJI bmyI
2201 TGAGAATGTT AAGGGCACTG AGGACTCAGG CACCACAGAC AAGAGAGTTG AGCTCAAAAC CCCACTTGGT GACACAACTC ACACATGCCC ACGGTGCCCA
     ACTCTTACAA TTCCCGTGAC TCCTGAGTCC GTGGTGTCTG TTCTCTCAAC TCGAGTTTTG GGGTGAACCA CTGTGTTGAG TGTGTACGGG TGCCACGGGT
                                              bsp1286
                                             nlaIV                                              bsp1286
     hgiJII                                  hgiCI     hgiJII                                  nlaIV      hgiJII
     bsp1286                              dsaI bmyI    bsp1286                             dsaI hgiCI     bsp1286
     bmyI                            bsp1286  banI     bmyI                                bsaJI bmyI     bmyI
     banII       maeIII    mnlI      bmyI bsaJI        banII        maeIII    mnlI    nlaIII    banI      banII
2301 GAGCCCAAAT CTTGTGACAC ACCTCCCCCG TGCCCACGGT GCCCAGAGCC CAAATCTTGT GACACACCTC CCCCATGCCC ACGGTGCCCA GAGCCCAAAT
     CTCGGGTTTA GAACACTGTG TGGAGGGGGC ACGGGTGCCA CGGGTCTCGG GTTTAGAACA CTGTGTGGAG GGGGTACGGG TGCCACGGGT CTCGGGTTTA
```

FIG. 6H

```
                                                                                       eam1105I
                                                                                     sau96I
                                                                             scrFI
                                               bsp1286                       mvaI    avaII
                                             nlaIV                           ecoRII
                                             hgiCI                           dsaV
                                         dsaI bmyI                           bstNI   asuI       mboII mboII
                                         bsaJI                               bsaJI mnlI        bpuAI earI/ksp632I      styI
     maeIII   mnlI   nlaIII    banI   alwNI                                  apyI[dcm+]        bbsI mnlI               bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

             sau96I
             nlaIV
             avaII
             asuI              maeII
           mspI               pmlI
           hpaII              eco72I                                          sau96I
          scrFI      mnlI    bsaAI                               drdI        avaII                maeII
          nciI     ddeI maeIII                               mboII      mnlI                    bsaAI
          dsaV    eco81I   bbrPI                             bpuAI    bsaJI    bsrI            rsaI
          cauII   bsu36I/mstII/sauI            maeII         bbsI   avaI  asuI                 csp6I              mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                    aciI
                   thaI
                   fnuDII/mvnI
                   bstUI
                   bsh1236I
                  sacII/sstII
                  nspBII                                                                 scrFI
                  kspI                                                                   mvaI bsrI
                  dsaI                                                                   ecoRII
                  bsaJI                                                                  dsaV
                  aciI                                                mnlI      econI    bstNI                  rsaI
                 fnu4HI  mnlI              maeII                  hgaI    hphI   bslI     apyI[dcm+]            csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6I

```
                                                                                                    rsaI
                bsmAI                                                                               csp6I
               bsaI        mnlI           taqI                                   avaI               bsp1407I    bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                          aciI
          bsaJI                   bstNI               bstNI                            dsaI           nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                       bslI          fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI           bspMI                                 bsaJI         bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                      dsaI
        mspI                                  pleI                 mnlI            hphI                 fnu4HI
        hpaII                     mnlI  nlaIII hinfI     nlaIV   mboII   scfI     aluI bsaJI        bspMI  bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                           scrFI
                                                                                                           nciI
                                                                                                           mspI
                                                                                                           hpaII
                                                                                                           dsaV
                                                                                       scrFI               cauII
                                                                                       nciI                bsaJI
                                                                                       mspI              haeIII/palI
                                   nlaIII                                              hpaII            mcrI
                                ppu10I                             sapI                dsaV             eagI/xmaIII/eclXI
         xmnI mboII             nsiI/avaIII                        mboII  mnlI      bsmAI               eaeI
         asp700      nlaIII    sfaNI   mnlI           aciI         earI/ksp632I bslI  cauII               cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
          rmaI
       mnlI
    sau3AI
    moI/ndeII[dam-]                         styI
    dpnI[dam+]                           aciI
    dpnII[dam-]  taqI                   fnu4HI   sau96I
    alwI[dam-]  salI    scfI           sfiI ncoI haeIII/palI
   nlaIV   maeI  hincII/hindII         haeIII/palI
   bstYI/xhoII  accI    pstI          eaeI  dsaI asuI               aluI
   bamHI xbaI pleI      bsgI    aluI cfrI   bsaJI                 fnu4HI
   alwI(dam-) hinfI bspMI    hindIII bglI nlaIII                bbvI          maeIII        sfaNI    apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                              sau3AI
                                                                                              mboI/ndeII[dam-]
                                                                                              dpnI[dam+]
                                                                                              dpnII[dam-]
                                                                                             pvuI/bspCI
                                                                                             mcrI
                                                                                           taqI[dam-]    tru9I
                                                                                          claI/bsp106[dam-]
                                                                                         sau3AI         mseI
                                                                                         mboI/ndeII[dam-]
                                                                                         dpnI[dam+] xmnI        hinPI
                                           rmaI                                          dpnII[dam-]  aseI/asnI/vspI
                                     bsmI  maeI                                 nlaIII  alwI[dam-]  asp700       hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                    rsaI
                                                    csp6I
        haeI                                       nlaIV
      styI                                         kpnI
      ncoI                                         hgiCI
      dsaI haeIII/palI                             banI                     aluI
  fnu4HI nlaIII                                    asp718     mnlI         pvuII
  bbvI  bsaJI               mnlI       mnlI        acc65I   ddeI aciI      nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

```
                 nlaIV                                                                          nlaIV
             scrFI                                                  scrFI        scrFI
             mvaI                                 ppu10I                  mvaI               mvaI
             ecoRII                          nsiI/avaIII            ecoRII       ecoRII
             dsaV                           nlaIII                  dsaV         dsaV
             bstNI                            sphI                        bstNI              bstNI
             apyI[dcm+]                       nspI sfaNI                  apyI[dcm+]         apyI[dcm+]
            bsaJI                             nspHI                      sexAI             bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                     sfaNI
                   ppu10I
                   nsiI/avaIII
                  nlaIII
                 sphI
                 nspI                                                      aciI                            bslI
                 nspHI                              aciI         aciI   fokI            aciI bsrI  aciI        aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                              rmaI
                                                                                                             styI
                                                                                                             bsaJI
                                                   fnu4HI                                                    blnI
        nlaIII                                    sfiI mnlI                                                  avrII
       styI                                       haeIII/palI                                              haeIII/palI
       ncoI                                  bsaJI bglI          ddeI                                     stuI
       dsaI                                haeIII/palI bsaJI mnlI  aluI                   mnlI           haeI
       bsaJI                             mnlI  mnlI aciI    haeIII/palI                mnlI             mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                              scrFI
                                                                              mvaI
                                                                              ecoRII
                                                                              dsaV
                       tru9I             haeIII/palI                          bstNI
                      hpaI              eaeI
                      hincII/hindII     cfrI                      bsrI        apyI[dcm+]          tru9I       fnu4HI
                  aluI mseI aluI     bsrI               maeII maeIII         bsaJI   maeIII      mseI        bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6L

```
                                                                                                     hinPI
                                                                                                      hhaI/cfoI
                                                                                                     nlaIV
                                                   sau3AI                                            narI
                                         sau96I    mboI/ndeII[dam-]                                  kasI
                                         haeIII/palI                                                 hinlI/acyI
                                         asuI      dpnI[dam+]                                        hgiCI
                    aluI               mnlI        dpnII[dam-]                                       haeII      aciI
                   pvuII            mboII   aciI  pvuI/bspCI                                         banI    sfaNI
     fokI          nspBII           earI/ksp632I  mcrI                          bglI                 ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                        aciI
                                                                                                     fnu4HI
                                                                            hinPI                    aciI
                                                                           thaI                     thaI
                                                                           fnuDII/mvnI              fnuDII/mvnI
                                                                           bstUI scfI     hinPI     bstUI
                                                                           bsh1236I       hhaI/cfoI hinPI
                                                                       rsaI hhaI/cfoI  fnu4HI  tru9I bsh1236I
                   sfaNI    aciI         aciI   maeII                  csp6I    bslI   aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

         fnu4HI
       hinPI
       hhaI/cfoI                             hinPI
      thaI                                   hhaI/cfoI
      fnuDII/mvnI                         rmaI                                                      mspI
      bstUI                          hinPI  haeII                                                   hpaII
      bsh1236I     aciI              hhaI/cfoI    bsrBI                                            naeI
     maeIII bbvI  maeIII            haeII maeI    aciI            mboII                   maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                    nlaIV
                   hgiJII
                   bsp1286                                 nlaIV                                      maeII    haeIII/palI
                   bmyI                                    hgiCI  taqI                               draIII  sau96I
      aluI         banII        nlaIV                      banI mnlI                    hphI         bsaAI   asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

```
                                         maeII pleI          tru9I     pleI                               bslI
                                        drdI   hinfI  maeII  mseI      hinfI        bsrI                  bslI  avaI
4201 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
     TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

                                                                                                thaI
                                                                                                fnuDII/mvnI
                                                              tru9I                       tru9I   apoI tru9I     tru9I
                                                              mseI             tru9I      mseI bstUI   mseI      mseI
                                                   haeIII/palI          aluI   mseI   apoI    bsh1236I        sspI
4301 GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
     CCGATAAGAA AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT GCGCTTAAAA TTGTTTTATA

                                hgiAI/aspHI
                                bsp1286
                                bsiHKAI                                                                         hinPI
                                bmyI    ddeI                  aciI                               maeIII       fnu4HI
         maeII                  apaLI/snoI    rsaI           fnu4HI     tru9I                 maeII bsrI     nlaIII hhaI/cfoI
        psp1406I                alw44I/snoI   csp6I       sfaNI         mseI       aciI      bsaAI tth111I/aspI bbvI
4401 TAACGTTTAC AATTTTATGG TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAACTCCG CTATCGCTAC GTGACTGGGT CATGGCTGCG
     ATTGCAAATG TTAAAATACC ACGTGAGAGT CATGTTAGAC GAGACTACGG CGTATCAATT CGGTTGAGGC GATAGCGATG CACTGACCCA GTACCGACGC

                                                                                                   scrFI
                                        hinPI                          sfaNI                       nciI
                                        hhaI/cfoI                   mspI                           mspI
                                       thaI                         hpaII                          hpaII     nspI
                                       fnuDII/mvnI                 scrFI                           dsaV      nspHI
                                       bstUI                       nciI                       esp3I       fnu4HI
                               nspBII  bsh1236I                    dsaV fokI               maeIII bsmAI    bbvI
                  aciI         aciI  hgaI       drdI               cauII   aciI        aluI    bslI   cauII aluI nlaIII
4501 CCCCGACACC CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
     GGGGCTGTGG GCGGTTGTGG GCGACTGCGC GGGACTGCCC GAACAGACGA GGGCCGTAGG CGAATGTCTG TTCGACACTG GCAGAGGCCC TCGACGTACA

                                         thaI
                                         fnuDII/mvnI
                                         bstUI
                                         bsh1236I
                                        hinPI
                                        hhaI/cfoI                         mnlI
                                      thaI                              haeIII/palI
                                      fnuDII/mvnI       mboII           sau96I                              nlaIII
                                      bstUI mnlI        bpuAI           asuI                         tru9I  rcaI
        mnlI     hphI        hphI     bsh1236I          bbsI           eco0109I/draII                mseI   bspHI
4601 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GTATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT
     CAGTCTCCAA AAGTGGCAGT AGTGGCTTTG CGCGCTCCGT CATAAGAACT TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT TACAGTACTA
```

FIG. 6N

```
                                                      nlaIV
                                                    aciI
                                                   thaI
                                                   fnuDII/mvnI
              hinlI/acyI                           bstUI                                                        rcaI
              ahaII/bsaHI                          bsh1236I                                                     bspHI
              aatII                               hinPI                                                     bsrBI
          ddeI maeII                              hhaI/cfoI                                                 aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                 mboII                                                      fnu4HI
       bsmAI                          sspI       earI/ksp632I                                               aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                                hgiAI/aspHI
                                                                                bsp1286
                                                                      sau3AI    bsiHKAI                       sau3AI
                                                                      mboI/ndeII[dam-]                        mboI/ndeII[dam-]
                                                                      dpnI[dam+] bmyI                         dpnI[dam+]
                                                                      dpnII[dam-]                            bstYI/xhoII
                                                                 eco57I         apaLI/snoI                bsrI dpnII[dam-]
                   hphI             hphI            sfaNI  mboII[dam-]          alw44I/snoI  maeIII taqI       alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                                 aciI
         sau3AI                                                                                 thaI
         mboI/ndeII[dam-]           maeII                                                       fnuDII/mvnI
         dpnI[dam+]                psp1406I             hgiAI/aspHI                             bstUI
         dpnII[dam-]               xmnI                 bsp1286 tru9I                           bsh1236I            hinlI/acyI
  aciI   alwI[dam-]                asp700               bsiHKAI mseI                           hinPI               hgaI
nspBII  bstYI/xhoII              mboII                  bmyI   ahaIII/draI                     hhaI/cfoI           ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

 scrFI
 nciI
 mspI
 hpaII                                                             rsaI
 dsaV                      aciI                                    csp6I   bsrI
 cauII      bcgI  mcrI  fnu4HI          ddeI                      scaI  hphI  maeIII      sfaNI     fokI    nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6O

```
                                                                                      sau96I
                                                                                      avaII
                                                                              sau3AI  asuI
                                                       haeIII/palI            mboI/ndeII[dam-]
                                                      eaeI                    dpnI[dam+]
                                                      cfrI                    dpnII[dam-]
                       fnu4HI                        fnu4HI                  pvuI/bspCI
                       bbvI        nlaIII            aciI                    mcrI   mnlI        aluI   aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                         maeIII
                      nlaIII
                   sau3AI                           mspI
                   mboI/ndeII[dam-]    sau3AI  nlaIV
                   dpnI[dam+]          mboI/ndeII[dam-] aluI
                   dpnII[dam-]         dpnI[dam+]   hpaII                                                  fnu4HI
           nlaIII alwI[dam-]           dpnII[dam-] bsaWI                                maeIII     sfaNI   bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                               mspI
                    hinPI                                      hpaII
                   mstI                                       scrFI
                   aviII/fspI     bsrI                  aluI  nciI        tru9I      fokI                    sau96I
              maeII hhaI/cfoI  tru9I                  rmaI    dsaV        mseI    bsrI       aciI            avaII
             psp1406I          mseI                   maeI    cauII      aseI/asnI/vspI   mnlI               asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

                    bglI                                        mspI               thaI
                   sau96I                                       hpaII              fnuDII/mvnI            haeIII/palI
                   haeIII/palI                                 cfr10I              bstUI                 sau96I
            hinPI  asuI    mspI                             nlaIV hphI        bsmAI aciI       fnu4HI   nlaIV
            hhaI/cfoI      hpaII                         gsuI/bpmI           bsaI  bsh1236I    bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                        ddeI
                                                                                    sau3AI       nlaIV
                                               pleI                                 mboI/ndeII[dam-] mnlI
                                               hinfI                                dpnI[dam+]   hgiCI         tru9I
             mnlI                        eam1105I               fokI                dpnII[dam-]  banI          mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```

FIG. 6P

```
                                                                                       rmaI      sau3AI
                                                                                    sau3AI hphI  mboI/ndeII[dam-]
                                                                                    mboI/ndeII[dam-]
                                                                                    dpnI[dam+]   dpnI[dam+]
                                                                                    dpnII[dam-]  dpnII[dam-]
                                                       tru9I             ahaIII/draI  maeI       alwI[dam-]
                                                       mseI          tru9I  tru9I bstYI/xhoII  bstYI/xhoII
          maeIII                                      ahaIII/draI    mseI   mseI  alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                  sau3AI
                                                                                  mboI/ndeII[dam-]
                                                                                  dpnI[dam+]  sau3AI
                                                                                  dpnII[dam-] mboI/ndeII[dam-]
                                                                                 bstYI/xhoII  dpnI[dam+]
                                                                          sau3AI  alwI[dam-]  dpnII[dam-]
         nlaIII            maeII                                          mboI/ndeII[dam-]    alwI[dam-]
        rcaI            tru9I                     hgaI                    dpnI[dam+] mboII[dam-]
        bspHI           mseI                   ddeI                       dpnII[dam-]        bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                sau3AI
      thaI                                                      mboI/ndeII[dam-]
      fnuDII/mvnI                                               dpnI[dam+]
      bstUI                                                     dpnII[dam-]
      bsh1236I                                                 alwI[dam-]
     hinPI     fnu4HI                          aciI           mspI                                bsrI
     hhaI/cfoI  bbvI                     aciI  nspBII         hpaII      aluI                   maeIII  eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

           hinPI                     rmaI              haeIII/palI
           hhaI/cfoI                 maeI     bslI    haeI                 scfI    aciI       mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                         scrFI                                      aciI
                                                         nciI                                      nspBII
                           fnu4HI                        mspI                         mspI        fnu4HI
                  alwNI    bbvI                          hpaII                        hpaII       bbvI
                 bsrI  fnu4HI                            dsaV     pleI               bsaWI      hinPI mcrI
            maeIII     bbvI    bsrI                      cauII    hinfI           maeIII        hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

                  hgiAI/aspHI
                  bsp1286
                  bsiHKAI
                  bmyI                                                                          hinPI
                  apaLI/snoI                                                                    hhaI/cfoI
                  alw44I/snoI   aluI                          ddeI      scfI                   haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                             scrFI
                                                                             mvaI          scrFI
                                                                             ecoRII  mvaI
                            mspI                                             dsaV    ecoRII
                            hpaII                                            bstNI         dsaV
                            bslI   fnu4HI                   hinPI mnlI       bsaJI         bstNI
              aciI         bsaWI   aciI                     hhaI/cfoI   aluI apyI[dcm+]    apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                  haeIII/palI
                                                                                                fnu4HI
                                                                                                aciI
                                                                                               thaI bslI
                                                                                               fnuDII/mvnI
                                   taqI                            nlaIV                       bstUI
                   mnlI  drdI   hgaI           sfaNI             aciI                          bsh1236I       nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
  haeIII/palI
 haeI
scrFI
mvaI bslI
ecoRII                 nlaIII
dsaV                  nspI
bstNI          haeIII/palI  nspHI                        tfiI                                       bsrBI
apyI[dcm+]     haeI         aflIII                       hinfI                  aciI          aluI  aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                                  thaI
                                                                                                  fnuDII/mvnI
                                                                                                  bstUI
                                                                                                  bsh1236I
                                                                                                 hinPI
                                                                                                 hhaI/cfoI
                                                                                                thaI
                                                                                                fnuDII/mvnI
    fnu4HI               fnu4HI                              sapI  hinPI                        bstUI   haeIII/palI
    bbvI                 bbvI pleI                           mboII hhaI/cfoI                    bsh1236I          tru9I
  aciI                 hinPI  hinfI                          earI/ksp632I              mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
 fnu4HI      mcrI      hhaI/cfoI          mnlI   aciI      haeII                   aciI       aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                      scrFI
                                                                                                      mvaI
                                                                                                      ecoRII
                                                                                                      dsaV
     aluI                                                           tru9I                       nlaIV bstNI
    pvuII                                       hinPI               mseI       maeIII           hgiCI apyI[dcm+]
    nspBII                  bsrI      aciI      hhaI/cfoI         aseI/asnI/vspI mnlI           banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                                  tru9I
                                                                                                  mseI
                                                                                                 aseI/asnI/vspI
                mspI                         aciI                                               xmnI
                hpaII                      bsrBI                           aluI      nlaIII     asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT

>length: 6889
```

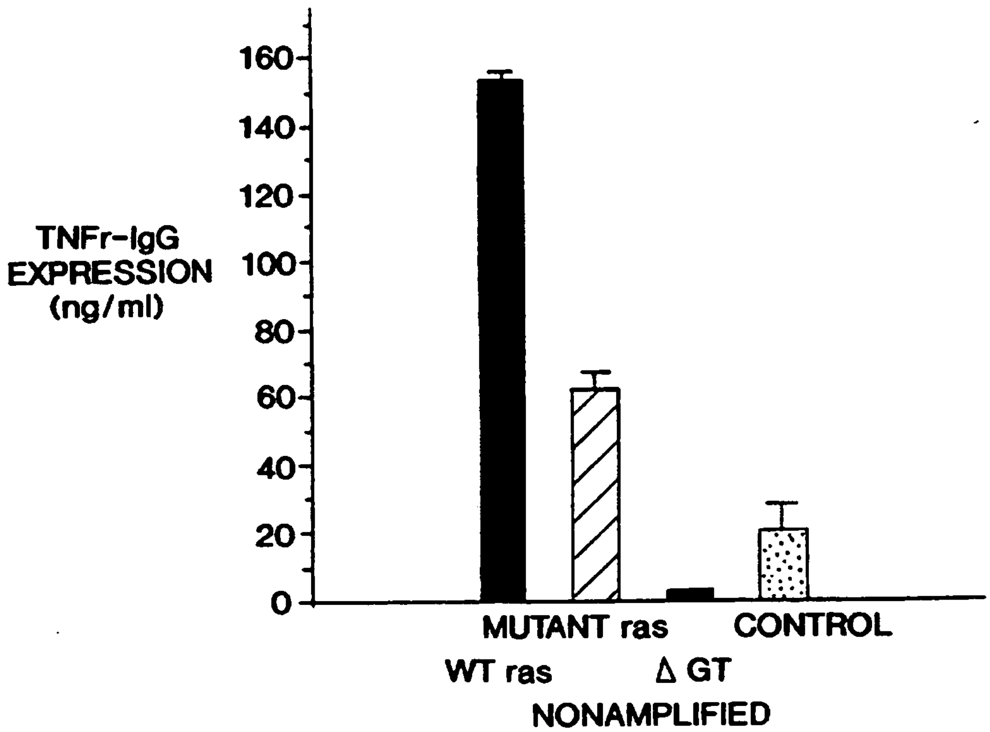

TNFr-IgG
EXPRESSION
(ng/ml)
160
140
120
100
80
60
40
20
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 7A

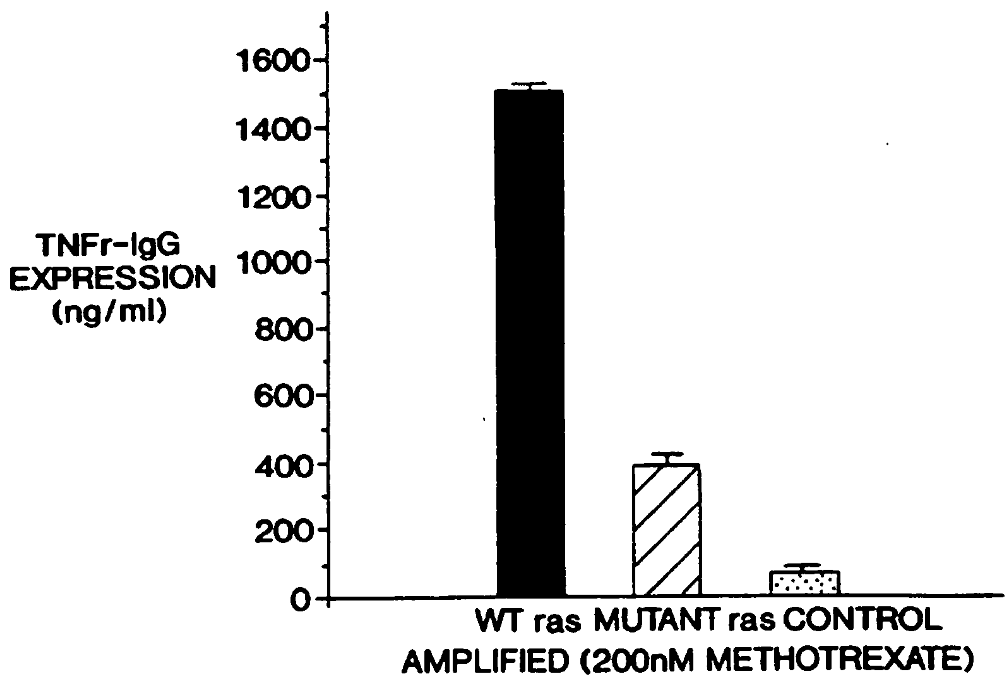

TNFr-IgG
EXPRESSION
(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

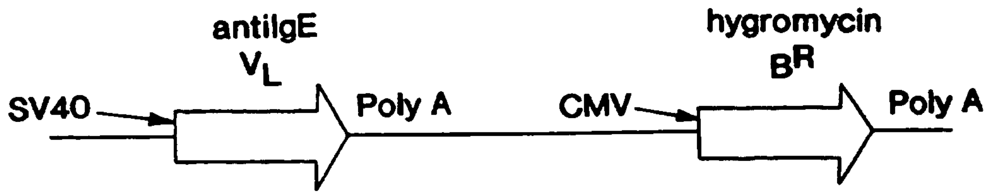

antiIgE
V_L
SV40
Poly A
CMV
hygromycin
B^R
Poly A

FIG. 11

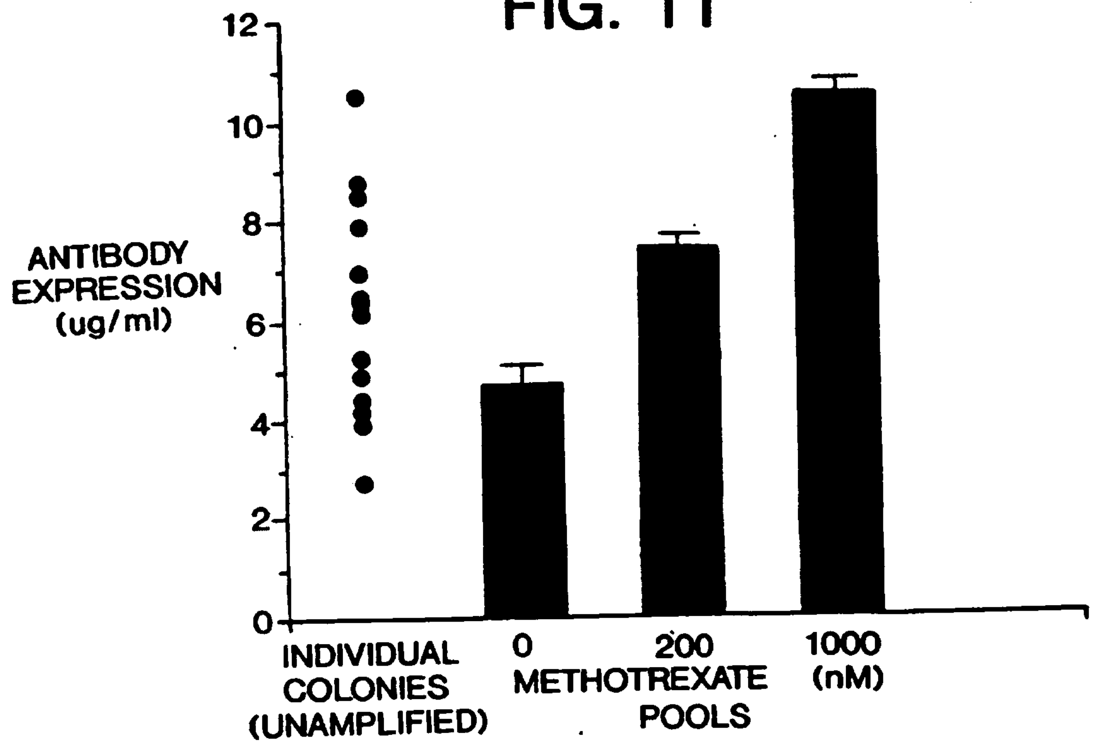

ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
METHOTREXATE POOLS
(nM)

FIG. 9A

```
       aluI
      sstI
      sacI                                         aluI
      hgiJII                               sau3AI pvuII                                  nlaIV
      hgiAI/aspHI                          mboI/ndeII[dam-]                          scrFI
      ecl136II                             dpnI[dam+]                                mvaI
      bsp1286                             pvuI/bspCI                                 ecoRII
      bsiHKAI                         pleI dpnII[dam-]                               dsaV
      bmyI                            hinfI  taqI[dam-]                              bstNI
      banII                       rmaI    mcrI    nspBII                             apyI[dcm+]
     taqI                         maeI   taqI[dam-]                                 bsaJI
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                                 nlaIV
                     sfaNI                 scrFI        scrFI                       sfaNI
                   ppu10I                  mvaI               mvaI                            ppu10I
                   nsiI/avaIII             ecoRII       ecoRII                    nsiI/avaIII
                  nlaIII                   dsaV         dsaV                     nlaIII
                 sphI                      bstNI              bstNI                         sphI
                 nspI                      apyI[dcm+]         apyI[dcm+]                    nspI
                 nspHI                    sexAI              bsaJI                          nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                       nlaIII
                                                                                      styI
                                                                                      ncoI
                                             aciI                                bslI dsaI
                      aciI         aciI  fokI                aciI bsrI   aciI    aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                                rmaI
                                                                               styI
                                                                               bsaJI                           haeIII/palI
                     fnu4HI                                                    blnI                           mcrI
                     bglI                                                      avrII                  aluI    eagI/xmaIII/eclXI
                    sfiI                                                     haeIII/palI            rmaI      eaeI
                    haeIII/palI                                             stuI                    maeI      cfrI
                  mnlI  mnlI      ddeI                           mnlI       haeI                   nheI      mspI
             haeIII/palI bsaJI  mnlI   aluI                  mnlI          mnlI maeI              aluI      hpaII
           mnlI bsaJI aciI    haeIII/palI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                              tfiI
    scrFI                     hinfI
    nciI                   aciI
    mspI                  thaI                                                                     fnu4HI
    hpaII                 fnuDII/mvnI                         aciI                                 bbvI
    dsaV                  bstUI                     maeII  rsaI                                 nspBII
    cauII                 bsh1236I               maeIII    csp6I  scfI            mnlI          aciI        nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                              haeIII/palI
                                                                             haeI
                                                                           scrFI
                                                                           mvaI     bsrBI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI    aciI                   rsaI
                                     pflMI                       bsmAI     apyI[dcm+]       xmnI          csp6I
    taqI           sfaNI             bslI                        bsaI     bsaJI  mnlI  ddeI  asp700       scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                        eco57I                                            dsaV
                       mboII                 tfiI                         bstNI                    tfiI       tru9I
                      earI/ksp632I           hinfI                        apyI[dcm+]               hinfI      mseI
                     mnlI                  alwNI   hphI                  sexAI            ddeI mboII  taqI   ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
             tru9I                                          bmyI                                   tru9I
             mseI         ddeI                              banII                                 aflII/bfrI
            aseI/asnI/vspI                       bslI   mnlI aluI           bstXI      fokI sfaNI  mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                               haeIII/palI
                                                                                              haeI
                                                                       scrFI         scrFI
                                                                       mvaI                mvaI
                                                                       ecoRII        ecoRII
         mspI                                                          dsaV   tfiI   dsaV
         hpaII                                                         bstNI    nlaIII     bstNI     ddeI pleI
        bsaWI                accI nlaIII             mnlI              apyI[dcm+]   hinfI  apyI[dcm+]    hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                     hgaI
                                                                                                    hinlI/acyI
                                                                                                    ahaII/bsaHI
                                                                                                 scrFI
                 nlaIII                                                                          mvaI     mnlI
             sau3AI                                                                              ecoRII
             mboI/ndeII[dam-]                                                                    dsaV
             dpnI[dam+]                  maeII                                                   bstNI    ecoNI
             dpnII[dam-]               aflIII                                                    apyI[dcm+]     mnlI
     maeIII alwI[dam-]  apoI       maeIII                                      mnlI            bsaJI     bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI[dcm+]
      sau96I
      avaII                                                           sfaNI
      asuI   mnlI       sfaNI                 accI     mboII             mboII                       mnlI     aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                     styI
                                                     bsaJI
                                               sau3AI
                       nlaIII                  mboI/ndeII[dam-]
                      styI                     dpnI[dam+]
                      ncoI                     dpnII[dam-]                 aluI     tru9I
     ppu10I           dsaI                     alwI[dam-]                fnu4HI     mseI
     nsiI/avaIII      bsaJI                   bstYI/xhoII                bbvI      aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9D

```
                                                                              sau96I
                                                                              avaII
                                                                              asuI
                                                                           scrFI
                                                                           mvaI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI                           ecoRI
           maeIII                                                          apyI[dcm+]       mnlI       taqI    apoI
          hphI    scfI         fokI                                 bslI  bsaJI            bsaJI     claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                        scrFI
        nlaIII                                                                                          mvaI
       styI                                                                                             ecoRII
       pflMI                                                                                            dsaV
       ncoI                                                                                             bstNI fnu4HI
       dsaI                                                            rsaI           aluI              apyI[dcm+]
       bslI fokI                          rmaI                  gsuI/bpmI           pvuII      pleI       haeI    bbvI
       bsaJI        nlaIII  fokI          maeI                 bsrI    csp6I        nspBII     hinfI   aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                                  scrFI
                                                                                                                  nciI
                                                                                                                  mspI
                                                                                                                  hpaII
                                                                                                                  dsaV
                                                                                                                  cauII
                                                                                                                  bslI
                                                                                                                 xmaI/pspAI
                                                                                                                 smaI
                                                                                                                 scrFI
                                                                                                                 nciI
                                                                                                                 dsaV
                                                                                                                 cauII        scrFI
     hgiJII                                                                                                   sau96I          mvaI
     bsp1286                                                                                          sau3AI  nlaIV           ecoRII
     bmyI                                                             mspI                            mboI/ndeII[dam-]        dsaV
scrFI                                                           hpaII                           dpnI[dam+]  bslI        bstNI
mvaI banII                                                           mroI                             dpnII[dam-]  bsaJI       bslI
ecoRII                                                         bspMII                          nlaIV        haeIII/palI    apyI[dcm+]
dsaV                                                           bspEI                           bstYI/xhoII    avaI     sau96I
bstNI                                                                bsaWI                           bamHI     eco0109I/draII haeIII/palI
bsaJI                                                              mnlI                              alwI[dam-] asuI          asuI
apyI[dcm+]                    alwNI                            hphI  accIII     aluI   bsrI alwI[dam-]  bsaJI      eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                              sau3AI
                              mboI/ndeII[dam-]
                              dpnI[dam+]                                                         thaI
                   maeII      dpnII[dam-]                                                        fnuDII/mvnI
             taqI snaBI      alwI[dam-]              hgaI      haeIII/palI                       bstUI  tfiI
            claI/bsp106     mspI                 xmaI          sau96I                            bsh1236I
        sfaNI    bsaAI      hpaII  taqI[dam-]     maeI          asuI                           nruI    hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

```
                                                                                       scrFI
                                                                                       mvaI
                                                                                       ecoRII
                                                                                       dsaV
                                                                                       bstNI
                                                                                       apyI[dcm+]
                                                                                    hinPI
                                                                                    hhaI/cfoI
                                                                mnlI               nlaIV
                                                             xhoI                  narI
                                                             paeR7I                kasI
                                                             avaI                  hinlI/acyI
       scfI                                              hgiAI/aspHI               hgiCI
       pstI                                              bsp1286                   haeII
       bsgI                     mnlI                     bsiHKAI   fnu4HI          banI
     bspMI                    ddeI drdI                  bmyI taqI bbvI            ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                             sau96I
                                             haeIII/palI
                                            sau96I                                                           scrFI
                                            nlaIV                                                            mvaI
                                            hgiJII                nlaIV                                      ecoRII
                                            bsp1286               hgiCI                                      dsaV
           scrFI                      bsp120I               banI                                       bstNI
           mvaI                             bmyI               scrFI                                   bsaJI
           ecoRII                     banII                mvaI                                     sau96I
           dsaV                       asuI                 ecoRII                              haeIII/palI
           bstNI hphI                       apaI        mboII   dsaV          hgiAI/aspHI           asuI
           apyI[dcm+] bsmAI    haeIII/palI eco0109I/draII   bstNI               bsp1286                 fnu4HI
          bsaJI maeIII    mnlI         styI asuI      bpuAI   apyI[dcm+]  mnlI    bsiHKAI      bsp1286 aciI apyI[dcm+]
      nlaIV   bstEII esp3I bsaJI mnlI  bsaJI          bbsI   bsaJI     mnlI       bmyI mnlI    bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9G

```
       scrFI                                                   hinPI
       mvaI                                                         nlaIV
       ecoRII                                                narI
       ecoNI                                                      kasI                 hgiAI/aspHI
       dsaV                    hphI                          hinlI/acyI          bsp1286     mspI
       bstNI                       mspI                           hgiCI                bsiHKAI     hpaII
       bslI                        hpaII                          haeII                bmyI       scrFI
       apyI[dcm+]                cfr10I                           banI           fnu4HI           nciI
    fnu4HI                       bsaWI  tth111I/aspI              ahaII/bsaHI    aciI  apaLI/snoI dsaV
    bbvI                   bslI  ageI maeIII                   ddeI hhaI/cfoI  nspBII alw44I/snoI cauII    scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                                 nlaIV
    ddeI pleI                                              fnu4HI  hgiCI
   mnlI  hinfI                                       mnlI  bbvI    banI                       tfiI
   eco81I            mnlI  fnu4HI  maeIII bsp1286 rmaI            bsp1286                    hinfI               styI
   bsu36I/mstII/sauI ddeI bbvI    hphI    bmyI    maeI aluI       bmyI                   maeII                   bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                          eam1105I
                                                                                         sau96I
                                                                                  scrFI
                                                                                  mvaI    avaII
                                                                                  ecoRII
                       hgiJII                                                     dsaV
                       bsp1286                      nlaIII                        bstNI   asuI       mboII mboII
                       bmyI                        nspI       bsp1286             bsaJI  nlaIV     bpuAI earI/ksp632I
                       banII          maeIII       nspHI      bmyI  alwNI         apyI[dcm+]       bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                    sau96I
                                    nlaIV
                                    avaII
                                  mspI
                                 scrFI
                                 nciI
                            sau3AI hpaII
                            mboI/ndeII[dam-]
                            dpnI[dam+]                   nlaIII
                         nlaIII    dsaV          mnlI   nspI                               drdI  mnlI
                        rcaI       cauII       ddeI maeIII                             mboII   ddeI
              styI      bspHI[dam-]  asuI     eco81I    nspHI                          bpuAI   eco81I
              bsaJI   mnlI  dpnII[dam-]      bsu36I/mstII/sauI       maeII             bbsI    bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                   aciI
                                                  thaI
                                                  fnuDII/mvnI
                                                  bstUI
                                                  bsh1236I
                                                 sacII/sstII
                                                 nspBII
                                                 kspI
       maeII                                     dsaI                              rsaI
      rsaI                                       bsaJI                             csp6I
      csp6I                                      aciI              rsaI          maeII                       mnlI      ecoNI
     bsrI bsaAI           mnlI                  fnu4HI   mnlI      csp6I        bsaAI                    hgaI   hphI   bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

     scrFI
     mvaI bsrI
     ecoRII
     dsaV
     bstNI                   rsaI          bsmAI                                                              fnu4HI
     apyI[dcm+]              csp6I        bsaI            mnlI          taqI                                  bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                           scrFI
                                           nciI
                                           mspI
                                           hpaII
                                           dsaV
                                           cauII
                                          xmaI/pspAI
                                          smaI
                                          scrFI                   scrFI
                                          nciI                    mvaI                scrFI
                                          dsaV                    ecoRII        mvaI
                                          cauII                   dsaV          ecoRII
                       rsaI            fokI    mboII              bstNI               dsaV
                       csp6I       bslI  bsaJI                    apyI[dcm+]          bstNI
      avaI            bsp1407I     bslI   avaI earI/ksp632I      sexAI          bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

           dsaI                         mspI
           bslI                    fnu4HI                                      pleI                  mnlI
           bsaJI                   bbvI hpaII                     mnlI         hinfI     nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                             bpuAI
                                             bbsI                   nlaIII
          dsaI                             maeII                 ppu10I                            sapI
          bsaJI                fnu4HI      xmnI mboII            nsiI/avaIII                       mboII  mnlI
       hphI            bspMI   bbvI        asp700    nlaIII     sfaNI   mnlI                       earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                   styI
           scrFI                           taqI                 aciI
           nciI                           salI                 fnu4HI   sau96I
           mspI                     rmaI  hincII/hindII       sfiI ncoI haeIII/palI
           hpaII                    maeI  accI   scfI         haeIII/palI
           dsaV                 sau96I  pleI     pstI        eaeI  dsaI asuI                 aluI
           cauII                haeIII/palI      bsgI   aluI cfrI  bsaJI                  fnu4HI
       bsmAI                    asuI    hinfI bspMI    hindIII bglI nlaIII                bbvI           maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                         taqI[dam-]
                                                                                                        claI/bsp106[dam-]
                                                                                                       sau3AI
                                                                                                       mboI/ndeII[dam-]
                                                                                                       dpnI[dam+]
                                                            rmaI                                       dpnII[dam-]
              sfaNI   apoI                             bsmI maeI                               nlaIII  alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

                tru9I                                                     rsaI
            xmnI                                                          csp6I
      sau3AI  aseI/asnI/vspI     styI                                    nlaIV
      mboI/ndeII[dam-]           ncoI                                    kpnI
      dpnI[dam+]           fnu4HI   haeI                                 hgiCI
      dpnII[dam-]          bbvI  dsaI haeIII/palI                        banI                        aluI
     pvuI/bspCI mseI     hinPI     bsaJI                                 asp718    mnlI             pvuII
     mcrI   asp700       hhaI/cfoI nlaIII          mnlI     mnlI         acc65I  ddeI aciI          nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                   nlaIV
                               scrFI                                                       scrFI
                               mvaI                                 ppu10I                      mvaI
                               ecoRII                          nsiI/avaIII                 ecoRII
                               dsaV                           nlaIII                       dsaV
                               bstNI                              sphI                          bstNI
                               apyI[dcm+]                         nspI sfaNI                    apyI[dcm+]
                              bsaJI                               nspHI                       sexAI          bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

        nlaIV                            sfaNI
     scrFI                         ppu10I
     mvaI                                nsiI/avaIII
     ecoRII                       nlaIII
     dsaV                        sphI
     bstNI                            nspI                                                       aciI
     apyI[dcm+]                       nspHI                      aciI          aciI   fokI             aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

```
                                                                     fnu4HI
                          nlaIII                                     sfiI mnlI
                          styI                                       haeIII/palI
                          ncoI                                  bsaJI bglI          ddeI
                    bslI  dsaI                                haeIII/palI bsaJI  mnlI  aluI              mnlI
     bsrI  aciI     aciI  bsaJI                             mnlI  mnlI aciI   haeIII/palI              mnlI
3201 CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
     GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC

                                                       fnu4HI            hinPI
                 rmaI                                mcrI                hhaI/cfoI
                styI                                 eagI/xmaIII/eclXI  thaI
                bsaJI                                eaeI               fnuDII/mvnI
                blnI                              bsrBI          tru9I  bstUI                 bspMI
                avrII                          xhoI notI      tru9I     bsh1236I           scfI
              haeIII/palI                      paeR7I haeIII/palI      hinPI      tru9I  pstI
             stuI                              avaI fnu4HI    pacI     hhaI/cfoI mseI    bsgI
             haeI                        maeIII taqI cfrI     mseI     bssHII  ahaIII/draI    maeIII
            mnlI maeI              aluI      mnlI  aciI aciI     mseI ascI   swaI    sse8387I    aluI    bsrI
3301 AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTGTTA CCTCGAGCGG CCGCTTAATT AAGGCGCGCC ATTTAAATCC TGCAGGTAAC AGCTTGGCAC
     TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGACAAT GGAGCTCGCC GGCGAATTAA TTCCGCGCGG TAAATTTAGG ACGTCCATTG TCGAACCGTG

                                       scrFI
                                       mvaI
                                       ecoRII
                                       dsaV
      haeIII/palI                      bstNI                                                 aluI
     eaeI                      bsrI    apyI[dcm+]         tru9I      fnu4HI                 pvuII           mboII
     cfrI              maeII maeIII   bsaJI    maeIII     mseI       bbvI  fokI             nspBII          earI/ksp632I
3401 TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCCTTC GCCAGCTGGC GTAATAGCGA
     ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT GAATTAGCGG AACGTCGTGT AGGGGGGAAG CGGTCGACCG CATTATCGCT

                                                             hinPI
                                                             hhaI/cfoI
                                                            nlaIV
                 sau3AI                                     narI
                 mboI/ndeII[dam-]                           kasI
                 dpnI[dam+]                                 hinlI/acyI
        sau96I   dpnII[dam-]                                hgiCI
        haeIII/palI                                         haeII     aciI
        asuI    pvuI/bspCI                                  banI   sfaNI
      mnlI aciI  mcrI                   bglI                ahaII/bsaHI              sfaNI   aciI
3501 AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGTAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA
     TCTCCGGGCG TGGCTAGCGG GAAGGGTTGT CAACGCATCG GACTTACCGC TTACCGCGGA CTACGCCATA AAAGAGGAAT GCGTAGACAC GCCATAAAGT
```

FIG. 9L

```
                                                    aciI
                    hinPI                          fnu4HI               fnu4HI
                    hhaI/cfoI                      aciI                hinPI
                   thaI                           thaI                 hhaI/cfoI
                   fnuDII/mvnI                    fnuDII/mvnI         thaI
                   bstUI           hinPI          bstUI               fnuDII/mvnI
                   bsh1236I        hhaI/cfoI hinPI                    bstUI                              hinPI
                rsaI        scfI fnu4HI    tru9I bsh1236I             bsh1236I    aciI                   hhaI/cfoI
   aciI  maeII  csp6I     bslI   aciI      mseI hhaI/cfoI        maeIII  bbvI  maeIII                    haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

      hinPI                                                                      nlaIV
      hhaI/cfoI                                     mspI                        hgiJII
     haeII                                          hpaII                       bsp1286
    rmaI    bsrBI                                  naeI                         bmyI
    maeI    aciI         mboII              maeII  cfr10I          aluI         banII       nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                nlaIV                                       maeII   haeIII/palI
                hgiCI  taqI                                draIII  sau96I                             maeII
                banI mnlI                       hphI       bsaAI   asuI                              drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

     pleI         tru9I     pleI                           bslI
     hinfI  maeII  mseI     hinfI          bsrI           bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                  thaI                                       hgiAI/aspHI
                                                  fnuDII/mvnI                                bsp1286
                                                  bstUI                   maeII              bsiHKAI
                 tru9I                         tru9I                   tru9I                 bmyI     ddeI
                 mseI               tru9I      mseI   apoI tru9I       mseI                  apaLI/snoI  rsaI
      haeIII/palI         aluI      mseI    apoI    bsh1236I mseI    sspI  psp1406I          alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                            hinPI
                                                                                                            hhaI/cfoI
                                                                                                           thaI
                                                                           hinPI                           fnuDII/mvnI
                      aciI                             maeIII          fnu4HI                              bstUI
                     fnu4HI    tru9I                 maeII bsrI nlaIII  hhaI/cfoI               nspBII     bsh1236I
                  sfaNI        mseI       aciI      bsaAI tth111I/aspI bbvI            aciI        aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                                                                                bstUI
                                                               scrFI                                            bsh1236I
                         mspI                                  nciI                                            hinPI
                         hpaII                                 mspI                                            hhaI/cfoI
                        scrFI                            bsmAI          nspI                                  thaI
                        nciI     aciI                   esp3I hpaII fnu4HI                                    fnuDII/mvnI
                        dsaV sfaNI                    bslI    dsaV aluI nspHI                                 bstUI
       drdI             cauII fokI      aluI maeIII           cauII bbvI nlaIII  mnlI   hphI      hphI        bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                    mnlI
                                  haeIII/palI                                                 maeII
                      mboII       sau96I                              nlaIII                 hinlI/acyI
                      bpuAI       asuI                          tru9I rcaI                   ahaII/bsaHI
       mnlI           bbsI       eco0109I/draII                 mseI  bspHI             ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                       nlaIV
                     aciI
                    thaI
                    fnuDII/mvnI
                    bstUI                                                     rcaI
                    bsh1236I                                                  bspHI
                   hinPI                                                 bsrBI   bsmAI
                   hhaI/cfoI                                             aciI nlaIII                        sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

                  mboII                                                 fnu4HI
                  earI/ksp632I                                          aciI                       hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                          hgiAI/aspHI
                                          bsp1286
                                sau3AI    bsiHKAI                          sau3AI  nspBII     sau3AI
                                mboI/ndeII[dam-]                           mboI/ndeII[dam-]   mboI/ndeII[dam-]
                                dpnI[dam+] bmyI                            dpnI[dam+]         dpnI[dam+]
                                dpnII[dam-]                              bstYI/xhoII          dpnII[dam-]
                           eco57I         apaLI/snoI                   bsrI dpnII[dam-]       alwI[dam-]
   hphI              sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-]    aciI  bstYI/xhoII          mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                      scrFI
                                                                      nciI
                                                   aciI               mspI
                                                  thaI                hpaII
                                                  fnuDII/mvnI         dsaV
      maeII               hgiAI/aspHI             bstUI               cauII
     psp1406I             bsp1286  tru9I          bsh1236I         hinlI/acyI
     xmnI                 bsiHKAI  mseI           hinPI            hgaI                          aciI
     asp700               bmyI   ahaIII/draI      hhaI/cfoI        ahaII/bsaHI    bcgI    mcrI   fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                                rsaI
                                csp6I    bsrI                                                  fnu4HI
       ddeI                    scaI hphI   maeIII      sfaNI     fokI    nlaIII                bbvI       nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                  sau96I
                                                  avaII                                  maeIII
                                            sau3AI  asuI                               nlaIII
                haeIII/palI                 mboI/ndeII[dam-]                         sau3AI
               eaeI                         dpnI[dam+]                               mboI/ndeII[dam-]  sau3AI
               cfrI                         dpnII[dam-]                              dpnI[dam+]        mboI/ndeII[dam-]
              fnu4HI                       pvuI/bspCI                                dpnII[dam-]       dpnI[dam+]
              aciI                         mcrI    mnlI        aluI   aciI      nlaIII alwI[dam-]      dpnII[dam-]
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9O

```
                                                                                               hinPI
       mspI                                                                                   mstI
       hpaII                                                                                  aviII/fspI    bsrI
       bsaWI                                                              fnu4HI         maeII hhaI/cfoI  tru9I
    nlaIV      aluI                                maeIII       sfaNI     bbvI           psp1406I         mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                  mspI
                  hpaII                                                                       bglI
                  scrFI                                                                       sau96I
             aluI nciI          tru9I      fokI                        sau96I                 haeIII/palI
           rmaI   dsaV          mseI   bsrI       aciI                 avaII          hinPI   asuI   mspI
           maeI   cauII         aseI/asnI/vspI  mnlI                   asuI           hhaI/cfoI      hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                   mspI            thaI
                   hpaII           fnuDII/mvnI            haeIII/palI
                   cfr10I          bstUI                 sau96I
              nlaIV  hphI       bsmAI aciI        fnu4HI  nlaIV
            gsuI/bpmI          bsaI bsh1236I      bbvI bsrI asuI             mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                              ddeI
                                           sau3AI       nlaIV
                                           mboI/ndeII[dam-]  mnlI
        pleI
        hinfI                              dpnI[dam+]   hgiCI        tru9I
     eam1105I            fokI              dpnII[dam-]  banI         mseI      maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                 rmaI     sau3AI
                                             sau3AI hphI  mboI/ndeII[dam-]
                                             mboI/ndeII[dam-]
                                             dpnI[dam+]   dpnI[dam+]
                                      tru9I  dpnII[dam-]  dpnII[dam-]
                tru9I               ahaIII/draI maeI      alwI[dam-]        nlaIII            maeII
                mseI            tru9I      bstYI/xhoII  bstYI/xhoII         rcaI            tru9I
              ahaIII/draI       mseI mseI  alwI[dam-] mboII[dam-]          bspHI            mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                                    sau3AI
                                                    mboI/ndeII[dam-]
                                                    dpnI[dam+]  sau3AI          thaI
                                                    dpnII[dam-] mboI/ndeII[dam-]
                                                   bstYI/xhoII  dpnI[dam+]      fnuDII/mvnI
                                           sau3AI  alwI[dam-]   dpnII[dam-]     bstUI
                                           mboI/ndeII[dam-]     alwI[dam-]      bsh1236I
                     hgaI                  dpnI[dam+] mboII[dam-]              hinPI       fnu4HI
                 ddeI                      dpnII[dam-]        bstYI/xhoII      hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                    sau3AI
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]
                                    dpnII[dam-]
                                   alwI[dam-]
                 aciI            mspI                                    bsrI            hinPI
       aciI   nspBII             hpaII      aluI                     maeIII   eco57I     hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                     fnu4HI
                                                                                             alwNI   bbvI
      rmaI               haeIII/palI                                                       bsrI   fnu4HI
      maeI      bslI    haeI                  scfI     aciI        mnlI                maeIII     bbvI    bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                      scrFI                                       aciI                       hgiAI/aspHI
                      nciI                                      nspBII                       bsp1286
                      mspI                         mspI        fnu4HI                        bsiHKAI
                      hpaII                        hpaII       bbvI                          bmyI
                      dsaV     pleI               bsaWI      hinPI mcrI                      apaLI/snoI
                      cauII    hinfI          maeIII         hhaI/cfoI                       alw44I/snoI   aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                     mspI
                                                            hinPI                                    hpaII
                                                            hhaI/cfoI                                bslI      fnu4HI
                          ddeI     scfI                    haeII                           aciI        bsaWI     aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                         scrFI
                                         mvaI          scrFI
                                         ecoRII  mvaI
                                         dsaV    ecoRII
                                         bstNI         dsaV
                       hinPI  mnlI       bsaJI         bstNI                                                  taqI
                       hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                           mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                  haeIII/palI
                                                              haeIII/palI     scrFI
                                                            fnu4HI            mvaI
                                                            aciI              ecoRII
                                                           thaI bslI          dsaV                    nlaIII
                                                           fnuDII/mvnI        bstNI bslI                   nspI
                               nlaIV                       bstUI              apyI[dcm+]    haeIII/palI    nspHI
            sfaNI            aciI                          bsh1236I      nlaIV haeI         haeI           aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                   fnu4HI              fnu4HI
                                                                                   bbvI                bbvI pleI
                          tfiI                                           bsrBI   aciI               hinPI   hinfI
                          hinfI                    aciI          aluI    aciI  fnu4HI       mcrI    hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                            thaI
                                                            fnuDII/mvnI
                                                            bstUI
                                                            bsh1236I
                                                          hinPI
                                                          hhaI/cfoI
                                                         thaI
                                                         fnuDII/mvnI
                      sapI  hinPI                        bstUI               tru9I   aluI
                      mboII hhaI/cfoI                    bsh1236I haeIII/palI       pvuII
                      earI/ksp632I               mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
            mnlI   aciI    haeII             aciI       aciI     cfrI  hinfI mseI   nspBII                   bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                                frusI                       nlaIV bstNI
                    hinPI       mseI      maeIII            hgiCI apyI[dcm+]              mspI
        aciI        hhaI/cfoI  aseI/asnI/vspI mnlI          banI  bsaJI                   hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                               tru9I
                                                               mseI
                                                              aseI/asnI/vspI
           aciI                                             xmnI
         bsrBI                          aluI      nlaIII    asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                   thaI
       bsiHKAI                                                                                   fnuDII/mvnJ
       bmyI                       rmaI     tru9I                                                 bstUI
       banII                      maeI     mseI                                                  bsh1236I
     taqI                        speI     aseI/asnI/vspI                             bslI       aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                  scrFI
                  mvaI
                  ecoRII
                  dsaV
               aciI
             bglI bstNI                                    maeII
            sau96I                                       hinlI/acyI
            haeIII/palI     aciI                         ahaII/bsaHI
            asuI  apyI[dcm+]               aciI          aatII         maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                    maeII
      hinlI/acyI                                                                               hinlI/acyI
      ahaII/bsaHI                                    rsaI                          rsaI        ahaII/bsaHI
      aatII                           bglI           csp6I           ndeI          csp6I       aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
       aciI                                                                                        nlaIII
     bglI  dsaV                                                                             styI
    sau96I bstNI          rsaI                                            maeII                   ncoI
    haeIII/palI           csp6I                                   rsaI    snaBI                   dsaI hphI  aciI
    asuI   apyI[dcm+]  bsrI  nlaIII                               csp6I   bsaAI                   bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                                           maeII
                                                                                           hinlI/acyI               nlaIV
               rsaI                          pleI                                          ahaII/bsaHI              hgiCI
               csp6I                  aciI   hinfI                  bsmAI                  aatII                    banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                            aluI
                                                                                                           sstI
                                                                                                           sacI
                                                                                                           hgiJII
                                                                                                           hgiAI/aspHI
                                                                                                           ecl136II
                                                                                                           bsp1286
                                                                                                           bsiHKAI
                                                                                 rsaI                      bmyI
                          maeIII     aciI       hgaI          aciI               csp6I      mnlI           banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                  haeIII/palI
                                                                                                 mcrI
                                                                                                 eagI/xmaIII/eclXI
                                                                                                 eaeI
                                                                                                 cfrI
                                                                                                fnu4HI
                                                                                                aciI
                                                                                               thaI
                      esp3I                                                                    fnuDII/mvnI
                 scrFI                                                 sau96I            sacII/sstII
                 mvaI bsmAI                                                  avaII             nspBII
                 ecoRII                                                asuI              kspI  scrFI
                 dsaV                                                 nlaIV              dsaI  nciI
                 bstNI  hinlI/acyI                                        scrFI            bglI bslI  mspI
                 apyI[dcm+]                                               nciI       sau3AI mnlI bstUI
            sau3AI gsuI/bpmI                                              mspI       mboI/ndeII[dam-] hpaII
            mboI/ndeII[dam-]                                       mboII  hpaII      dpnI[dam+] bsaJI dsaV
            dpnI[dam+]  hgaI  fokI                                 bpuAI  dsaV       dpnII[dam-] bsh1236I
            dpnII[dam-] ahaII/bsaHI                    mnlI        bbsI   cauII      alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
         tfiI
       aciI
      thaI hinfI
      fnuDII/mvnI                                                         fnu4HI
      bstUI                                                bstXI          aciI
      bsh1236I                              aciI         sau96I  styI     thaI
                                   maeII rsaI        pleI scfI haeIII/palI fnuDII/mvnI tru9I
                                maeIII    csp6I  scfI hinfI  asuI    bsaJI  bstUI  mseI
                                                                          bsh1236I  aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                             sau96I
                                                                             avaII
                                                                             asuI
                                                                           scrFI
                                                                           mvaI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI
                                  maeIII                                   apyI[dcm+]
                                 hphI   scfI       fokI                  bslI bsaJI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                              hinlI/acyI
                                              ahaII/bsaHI
                                              aatII
                                            thaI                taqI[dam-]
                                            fnuDII/mvnI        sau3AI             bsmAI
                  aluI  taqI                bstUI              mboI/ndeII[dam-]  esp3I
    mnlI         hindIII                   aciI maeII          dpnI[dam+]     tth111I/aspI
    bsaJI   ddeI     claI/bsp106       hphI bsh1236I    taqI    dpnII[dam-] taqI   hgaI        sfaNI
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                             hinPI
                                                                          fnu4HI
     aluI               tfiI                                              bbvI
    fnu4HI              hinfI                taqI                         aluI hhaI/cfoI
    bbvI       mnlI  mboII              aluI         mnlI           aciI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                         hinPI
                                         hhaI/cfoI    mspI
                                        thaI          hpaII
                                       aciI          mroI
                                    haeIII/palI      bspMII
                                   mcrI fnuDII/mvnI  bspEI
     sau3AI                        eagI/xmaIII/eclXI bsaWI
     mboI/ndeII[dam-]              eaeI bstUI    tfiI
     dpnI[dam+]                    cfrI bsh1236I hinfI                ecoRI
     dpnII[dam-]               sfaNI  fnu4HI  bslI  accIII            apoI                       sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                          mcrI
                                                                                                          eagI/xmaIII/eclXI
                                                                                                          eaeI
                                                                                       styI              fnu4HI
        hgiAI/aspHI                                                           thaI     ncoI       sau3AI aciI
        bsp1286                                                               fnuDII/mvnI         mboI/ndeII[dam-]
        bsiHKAI                                                     fnu4HI    bstUI    dsaI       dpnI[dam+]   sau3AI
        bmyI                                                        bbvI  mcrI      haeIII/palI   dpnII[dam-]  mboI/ndeII[dam-]
        apaLI/snoI                                                scfI   mspI bsh1236I bsaJI sfaNI    fnu4HI   dpnI[dam+]
        alw44I/snoI    maeII                             nspBII   pstI   hpaII    mnlI nlaIII  pvuI/bspCI haeIII/palI
      bslI draIII    maeIII       bspMI                  aciI     bsgI  cfr10I aciI haeI   fokI mcrI bbvI cfrI dpnII[dam-]
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                       sau96I                                              thaI      sau3AI
                                       avaII                                               fnuDII/mvnI     nlaIII
                                       asuI                                                bstUI     mboI/ndeII[dam-]
                             sau96I   rsrII/cspI                                           bsh1236I  dpnI[dam+]
                    aciI     haeIII/palI  aciI    tfiI                                    hinPI      dpnII[dam-]
      ddeI        bsrBI      asuI     cpoI        hinfI              nlaIII          ndeI hhaI/cfoI  alwI[dam-]
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                      hinPI                                                   draIII
                                                      hhaI/cfoI                                             nlaIV
                                                     thaI                                  bsaJI            hgiCI
                                                     fnuDII/mvnI                         haeIII/palI         banI
                               tth111I/aspI          bstUI                  sfaNI       sau96I             mspI
          bsrI            drdI            hgaI       bsh1236I   taqI   aluI             asuI mnlI    bslI  hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
                aciI
               thaI
               fnuDII/mvnI
         hgiAI/aspHI
         bsp1286
         bsiHKAI                                  aciI
         bmyI  bstUI                             fnu4HI
         apaLI/snoI                             haeIII/palI
         alw44I/snoI                           eaeI          aciI        gsuI/bpmI               tfiI bslI
     mnlI      bsh1236I   nlaIV                cfrI        nspBII      bsrI     mnlI             hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                         fnu4HI
                                                                                                        thaI
                                                                                                        fnuDII/mvnI
                                                                                                        bstUI
                                                                                                        bsh1236I
                                                                                                       sacII/sstII
                                                                                                       nspBII
                                                                                                       kspI
                                                                                     mspI              dsaI
                                                                                     hpaII             bsaJI
                                                                                    mroI               aciI
                                                                                    bspMII            fnu4HI
                                                                                    bspEI          sau3AI aciI
                                  dsaI                                              bsaWI          mboI/ndeII[dam-]
                               haeIII/palI                         rsaI     aciI   fokI            dpnI[dam+]
                      mboII  mnlI bsaJI               fnu4HI       csp6I  bsrBI   sfaNI    aluI    dpnII[dam-]
        mnlI      mboII   gsuI/bpmI                   bbvI   maeII   taqI    mnlI  accIII         alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

          scrFI
          nciI
          mspI
          hpaII
          dsaV
          cauII                                                                        aluI
                                                                                      fnu4HI          hgaI
                                                                                      bbvI     hinPI    taqI  drdI
      nlaIV           aciI                           aluI   hincII/hindII taqI  sfaNI     hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
                nlaIV
               mspI
               hpaII  scrFI                                                         haeIII/palI
               bslI   nciI                                                         mcrI
              mroI    mspI                                                         eagI/xmaIII/eclXI
              bspMII  hpaII                                                        eaeI
              bspEI[dam-]                                                          cfrI
              bsaWI   dsaV                                                        fnu4HI
              accIII[dam-]                                                        aciI
             sau3AI   cauII                                                      thaI
             mboI/ndeII[dam-]                                                    fnuDII/mvnI
             dpnI[dam+]                                                          bstUI
             dpnII[dam-]                                                         bsh1236I    sau96I                 rsaI
             dpnII[dam-]                  rsaI                                  hinPI        avaII                  csp6I
             alwI[dam-]                   csp6I          aciI                   hhaI/cfoI    asuI                  scaI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                                 scrFI
                                                                                 nciI
                                                                                 mspI
                                                                                 hpaII
                                                                                 dsaV
                                                                                xmaI/pspAI
                                                                                smaI
                                                                                scrFI
                                                                                nciI
                                                                                dsaV
                                                                                cauII
                                                                                bsaJI
                                                                                avaI
                                                                               bsaJI
                                                                             sau3AI                          sau96I
                                                                             mboI/ndeII[dam-]         aciI   haeIII/palI
                                                                             dpnI[dam+]              fnu4HI  asuI
                                                                             dpnII[dam-]             bglI  nlaIII
                                                                             alwI[dam-]             sfiI  styI
                                                                            nlaIV cauII            eaeI  ncoI
             hinlI/acyI                                          mcrI       bstYI/xhoII            cfrI  dsaI
             hgaI                    mnlI                       bslI        bamHI bsaJI ecoRI    taqI haeIII/palI
             ahaII/bsaHI          bsaJI                       sfaNI         alwI[dam-]  apoI    claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                     aluI
                   fnu4HI
                   bbvI          maeIII              sfaNI   apoI                              rmaI
                                                                                         bsmI  maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                            sau3AI
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]
                                            dpnII[dam-]
                                           pvuI/bspCI
                                           mcrI
                                          taqI[dam-]  tru9I                haeIII/palI
                                         claI/bsp106[dam-]                haeI
                                        sau3AI        mseI       fnu4HI styI
                                        mboI/ndeII[dam-]         bbvI   ncoI
                                        dpnI[dam+] xmnI        hinPI    dsaI
                                        dpnII[dam-]  aseI/asnI/vspI     bsaJI
                               nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

                                                                            nlaIV
      rsaI                                                               scrFI                            sfaNI
      csp6I                                                              mvaI                                ppu10I
     nlaIV                                                               ecoRII                         nsiI/avaIII
     kpnI                                                                dsaV                          nlaIII
     hgiCI                                                               bstNI                              sphI
     banI                        aluI                                    apyI[dcm+]                         nspI
     asp718     mnlI            pvuII                                   bsaJI                               nspHI
     acc65I  ddeI  aciI         nspBII
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                              nlaIV
                        scrFI        scrFI
                        mvaI               mvaI                           ppu10I
                        ecoRII       ecoRII                        nsiI/avaIII
                        dsaV         dsaV                         nlaIII
                        bstNI              bstNI                           sphI
                        apyI[dcm+]         apyI[dcm+]                      nspI  sfaNI
                       sexAI              bsaJI                            nspHI                          aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                               fnu4HI
                                                                                                               bglI
                                                                    nlaIII                                    sfiI
                                                                   styI                                       haeIII/palI
                                                                   ncoI                                     mnlI
                         aciI              bsrI               bslI dsaI                                 haeIII/palI
                aciI fokI              aciI       aciI        aciI bsaJI                             mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                               scrFI
                                                                                               nciI
                                                                                               mspI
                                                                                               hpaII
                                                                                               dsaV
                                                            styI                             haeIII/palI
                                                            bsaJI                          mcrI
                                                            blnI                           eagI/xmaIII/eclXI
                                                         haeIII/palI           rmaI        eaeI
          haeIII/palI                                   stuI rmaI              maeI        cfrI
      mnlI      ddeI                      mnlI          haeI maeI             nheI        mspI cauII
      bsaJI mnlI   aluI                mnlI            mnlI avrII            aluI aluI    hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

           tfiI
           hinfI                                                                             fnu4HI
        aciI                                                                                 aciI
       thaI                                                       bstXI                    thaI
       fnuDII/mvnI                         aciI          scfI  sau96I    styI              fnuDII/mvnI  tru9I
       bstUI                pleI        rsaI         pleI      haeIII/palI                 bstUI        mseI
       bsh1236I             hinfI       csp6I  scfI  hinfI     asuI      bsaJI             bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                  sau3AI
                  mboI/ndeII[dam-]
                  dpnI[dam+]
                  dpnII[dam-]
                  alwI[dam-]
                taqI[dam-]
              claI/bsp106[dam-]                                                   sau96I
             sau3AI                                                               avaII
             mboI/ndeII[dam-]                                                     asuI
             dpnI[dam+]                                                        scrFI
             dpnII[dam-]                                                       mvaI                  rmaI
            alwI[dam-]                                                         ecoRII      thaI    maeI
                                                                               dsaV        fnuDII/mvnI
                                                                               bstNI             bstUI  nheI
                                                                               apyI[dcm+]  mnlI  bsh1236I  aluI
                                   fokI                                bslI  bsaJI         bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                              nlaIII
                             styI
                             pflMI
                             ncoI
            sfaNI     ecoRI  dsaI                                                   rsaI              aluI
         fnu4HI taqI  apoI   bslI fokI                          rmaI         gsuI/bpmI           pvuII tth111I/aspI
         bbvI claI/bsp106    bsaJI      nlaIII  fokI            maeI         bsrI  csp6I    ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

            aluI
           sstI
           sacI
           hgiJII
           hgiAI/aspHI
           ecl136II
           bsp1286
           bsiHKAI
           bmyI                                     hphI
           banII          mnlI                    maeIII   bspMI                taqI                          bsrI
        avaI          aciI                       bstEII   hphI        bsrI     hgaI           hphI   aluI  nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                            mspI
                                                                                            hpaII
                                                                                            bslI
                                                          gsuI/bpmI                        bsaWI
                                                         scrFI                      sau3AI
                                                         mvaI                            mboI/ndeII[dam-]
                                                haeIII/palI                              dpnI[dam+]
                scrFI                    fnu4HI     ecoRII                    dpnII[dam-]
                mvaI                           aciI           dsaV                  alwI[dam-]
                ecoRII                  thaI mnlI   bstNI                          nlaIV
                dsaV                    fnuDII/mvnI apyI[dcm+] pleI                bstYI/xhoII
                bstNI    aluI                bstUI    rsaI  pleI gsuI/bpmI                  bamHI
                apyI[dcm+]                   bsh1236I csp6I hinfI  hinfI                    alwI[dam-]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                              sau3AI                   styI
                                                                              mboI/ndeII[dam-]         bsaJI
                                                                              dpnI[dam+]            rsaI
                                                                              dpnII[dam-]           csp6I
                                                                              alwI[dam-]           nlaIV
                                fnu4HI mboII                                 nlaIV                 kpnI
                                bbvI   bpuAI                                 bstYI/xhoII           hgiCI
                              scfI     bbsI                                  bamHI rsaI            banI
                              pstI   mspI                                  mnlI    csp6I           asp718
                              bsgI   hpaII                            maeIII alwI[dam-]            acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

             sau3AI
             mboI/ndeII[dam-]             mboII
             dpnI[dam+]      fnu4HI      bpuAI       aciI
             dpnII[dam-]     bbvI        bbsI    mboII                                  mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                            scrFI
                                                                            mvaI
                                                                            ecoRII
                                                                            dsaV
                            haeIII/palI                                     bstNI
       xmnI                haeI    rsaI                   mnlI              apyI[dcm+]
       asp700             mnlI     csp6I                  bslI      maeIII bsaJI      maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

```
                                                                                                               sstI
                                                                                                               sacI
                                                                                                               hgiJII
                                                                                                               hgiAI/aspHI
                                                                                                               ecl136II
                                                                                                               bsp1286
                                                                                                               bsiHKAI
                                                                                                               bmyI
                                                                                                          haeIII/palI
                                           ddeI                                                          sau96I aluI
                                          celII/espI                                                     asuI  banII
                       ddeI fnu4HI        bpu1102I                                            hphI      eco0109I/draII
                scfI  mnlI  bbvI       hgaI                                 accI             maeIII   alwNI  ddeI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                   sau96I
                                                                nlaIII
                                                            aciI   haeIII/palI
                                                          fnu4HI   asuI
                                                          bglI styI
                                               aluI      sfiI  ncoI
                                              hindIII   eaeI   dsaI                     aluI
                                            tru9I       cfrI   bsaJI                  fnu4HI
            maeIII    aluI                  mseI    taqI haeIII/palI                  bbvI          maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                               sau3AI
                                                                                                               mboI/ndeII[dam-]
                                                                                                               dpnI[dam+]
                                                                                                               dpnII[dam-]
                                                                                                              pvuI/bspCI
                                                                                                              mcrI
                                                                                                             taqI[dam-]
                                                                                                            claI/bsp106[dam-]
                                                                                                           sau3AI
                                                                                                           mboI/ndeII[dam-]
                                                                                                           dpnI[dam+]
                                                        rmaI                                               dpnII[dam-]
          sfaNI   apoI                             bsmI maeI                                      nlaIII  alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```

FIG. 10L

```
                                                                         rsaI
                                 haeIII/palI                             csp6I
                                haeI                                    nlaIV
           tru9I      fnu4HI styI                                       kpnI
           mseI       bbvI   ncoI                                       hgiCI
          aseI/asnI/vspI     dsaI                                       banI                       aluI
        xmnI        hinPI    bsaJI                                      asp718     mnlI           pvuII
        asp700      hhaI/cfoI nlaIII          mnlI      mnlI            acc65I  ddeI  aciI        nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                  nlaIV                                                                         nlaIV
                               scrFI                          sfaNI                 scrFI        scrFI
                               mvaI                               ppu10I                  mvaI               mvaI
                               ecoRII                       nsiI/avaIII             ecoRII       ecoRII
                               dsaV                        nlaIII                   dsaV         dsaV
                               bstNI                            sphI                      bstNI              bstNI
                               apyI[dcm+]                       nspI                      apyI[dcm+]         apyI[dcm+]
                              bsaJI                             nspHI                     sexAI             bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                    ppu10I
                                    nsiI/avaIII
                                   nlaIII
                                  sphI
                                  nspI sfaNI                                                                 bsrI
                                  nspHI                              aciI          aciI fokI aciI         aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                    fnu4HI
                                                                    bglI
                         nlaIII                                    sfiI
                        styI                                       haeIII/palI
                        ncoI                                     mnlI    mnlI      ddeI
                   bslI dsaI                                 haeIII/palI bsaJI mnlI   aluI                  mnlI
       aciI        aciI bsaJI                             mnlI bsaJI aciI    haeIII/palI                 mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
                styI                                                                                         scrFI
                bsaJI                                                                                        mvaI
                blnI                                                                                         ecoRII
             haeIII/palI                 tru9I              haeIII/palI                                      dsaV
            stuI rmaI                    mseI              eaeI                                              bstNI
            haeI maeI                   hpaI  aluI         cfrI                     maeIII                   apyI[dcm+]       tru9I
           mnlI avrII              aluI hincII/hindII     bsrI                maeII    bsrI                  bsaJI  maeIII    mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                           sau3AI
                                                                           mboI/ndeII[dam-]
                                                                 sau96I    dpnI[dam+]
                                                                 haeIII/palI
                                            aluI              mnlI  aciI   dpnII[dam-]
              fnu4HI                       pvuII            mboII asuI    pvuI/bspCI
              bbvI   fokI                  nspBII           earI/ksp632I  mcrI                   bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

      hinPI
      hhaI/cfoI
     nlaIV                                                                                   hinPI
     narI                                                                                    hhaI/cfoI
     kasI                                                                                   thaI
     hinlI/acyI                                                                             fnuDII/mvnI          hinPI
     hgiCI                                                                                  bstUI         hinPI   hhaI/cfoI
     haeII    aciI                                                                          bsh1236I      hhaI/cfoI
     banI  sfaNI                                                                         rsaI      scfI fnu4HI  tru9I
     ahaII/bsaHI                          sfaNI    aciI           aciI  maeII            csp6I   bslI  aciI    mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                          fnu4HI
        aciI            hinPI
      fnu4HI            hhaI/cfoI                                hinPI
      aciI             thaI                                      hhaI/cfoI
     thaI              fnuDII/mvnI                              haeII
     fnuDII/mvnI       bstUI                              hinPI rmaI
     bstUI             bsh1236I     aciI                  hhaI/cfoI    bsrBI
     bsh1236I     maeIII  bbvI maeIII                     haeII  maeI  aciI           mboII                maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                       nlaIV
      mspI                            hgiJII                                  mnlI
      hpaII                           bsp1286                             nlaIV
     naeI                             bmyI                                hgiCI
     cfr10I            aluI           banII         nlaIV                 banI   taqI                    hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

         maeII    haeIII/palI
        draIII   sau96I                                  maeII  pleI          tru9I     pleI
        bsaAI    asuI                                   drdI    hinfI maeII   mseI      hinfI         bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                             thaI
                                                                                                             fnuDII/mvnI
                                                                                                             bstUI
                                                                           tru9I                             bsh1236I
            bslI                                                           mseI              .tru9I      tru9I
           bslI   avaI                                          haeIII/palI           aluI    mseI  apoI mseI   apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                           hgiAI/aspHI
                                           bsp1286
                        maeII              bsiHKAI                                                        tth111I/aspI
                     tru9I                 bmyI    ddeI                    aciI                          maeIII
         tru9I       mseI                  apaLI/snoI  rsaI               fnu4HI    tru9I                maeII
         mseI    sspI  psp1406I            alw44I/snoI csp6I          sfaNI       mseI         aciI     bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                                    hinPI
                                                    hhaI/cfoI                 mspI
                                                   thaI                       hpaII
                   hinPI                           fnuDII/mvnI               scrFI                       bsmAI
                fnu4HI                             bstUI                     nciI     aciI                esp3I
                bbvI                       nspBII  bsh1236I                  dsaV sfaNI                  bslI
           nlaIII  hhaI/cfoI    aciI       aciI  hgaI       drdI             cauII fokI             aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                      thaI
                                                      fnuDII/mvnI
                                                      bstUI
                                                      bsh1236I
      scrFI                                          hinPI
      nciI                                           hhaI/cfoI                          mnlI
      mspI      nspI                                 thaI                             haeIII/palI
      hpaII fnu4HI                                   fnuDII/mvnI          mboII       sau96I
      dsaV aluI nspHI                                bstUI mnlI           bpuAI       asuI
      cauII bbvI nlaIII    mnlI   hphI      hphI     bsh1236I             bbsI      eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                          nlaIV
                                                                        aciI
                                                                       thaI
                                                                       fnuDII/mvnI
                                      maeII                            bstUI
        .        nlaIII              hinlI/acyI                        bsh1236I
       tru9I rcaI                    ahaII/bsaHI                      hinPI
       mseI  bspHI              ddeI aatII                            hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGATAAACA  AATAAAAAGA TTTATGTAAG

                 rcaI
                 bspHI
             bsrBI   bsmAI                                       mboII
             aciI nlaIII                          sspI           earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                           hgiAI/aspHI
                                                                                           bsp1286
                                                                                  sau3AI   bsiHKAI
                                                                                  mboI/ndeII[dam-]
                                                                                  dpnI[dam+] bmyI
                                                                                  dpnII[dam-]
                                                                             eco57I        apaLI/snoI
           fnu4HI                                                                                          
           aciI                    hphI             hphI             sfaNI mboII[dam-]     alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
             sau3AI                                                                    aciI
             mboI/ndeII[dam-]    sau3AI                                               thaI
             dpnI[dam+]          mboI/ndeII[dam-]                                     fnuDII/mvnI
             dpnII[dam-]         dpnI[dam+]               psp1406I      hgiAI/aspHI   bstUI
            bstYI/xhoII          dpnII[dam-]              xmnI          bsp1286 tru9I bsh1236I
         bsrI          nspBII    alwI[dam-]               asp700        bsiHKAI mseI  hinPI
      taqI  alwI[dam-]  aciI    bstYI/xhoII           mboII maeII       bmyI  ahaIII/draI hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                scrFI
                nciI
                mspI
                hpaII
                dsaV
                cauII
              hinlI/acyI                                               rsaI
              hgaI                aciI                                 csp6I bsrI
              ahaII/bsaHI  bcgI  mcrI fnu4HI        ddeI               scaI hphI  maeIII    sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                          sau96I
                                                                                          avaII
                                                                                  sau3AI  asuI
                                                              haeIII/palI         mboI/ndeII[dam-]
                                                              eaeI                dpnI[dam+]
                                                              cfrI                dpnII[dam-]
                                  fnu4HI                      fnu4HI              pvuI/bspCI
       fokI  nlaIII               bbvI        nlaIII          aciI                mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                 nlaIII
                              sau3AI maeIII                      mspI
                              mboI/ndeII[dam-]   sau3AI   nlaIV
                              dpnI[dam+]         mboI/ndeII[dam-]
                             alwI[dam-]          dpnI[dam+]   hpaII
     aluI   aciI          nlaIII dpnII[dam-]     dpnII[dam-] bsaWI aluI                            maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                            mspI
                                         hinPI                              hpaII
                                       mstI                                scrFI
                                       aviII/fspI       bsrI           aluI nciI         tru9I       fokI
                 fnu4HI              maeII hhaI/cfoI tru9I            rmaI  dsaV         mseI     bsrI        aciI
          sfaNI  bbvI               psp1406I         mseI             maeI  cauII      aseI/asnI/vspI    mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                              aciI
                                         bglI                                     mspI       thaI
                                       sau96I                                     hpaII      fnuDII/mvnI
                   sau96I              haeIII/palI                               cfr10I      bstUI
                   avaII        hinPI asuI    mspI                             nlaIV  hphI  bsmAI          fnu4HI
                   asuI         hhaI/cfoI     hpaII                          gsuI/bpmI     bsaI bsh1236I  bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                   ddeI
              sau96I                                                                             sau3AI
              asuI                                             pleI                              mboI/ndeII[dam-]
             nlaIV                                             hinfI                             dpnI[dam+]
          bsrI haeIII/palI       mnlI                    eam1105I              fokI              dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                      hphI
                                                                                                     rmaI
                                                                                                   sau3AI
                                                                                                   mboI/ndeII[dam-]
                                                                                                   dpnI[dam+]
           mnlI                                                                          tru9I     dpnII[dam-]
      nlaIV                                                              tru9I          ahaIII/draI maeI
      hgiCI          tru9I                                               mseI        tru9I      bstYI/xhoII
      banI           mseI        maeIII                                 ahaIII/draI  mseI mseI  alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
                                                                                                         sau3AI
                                                                                                         mboI/ndeII(dam-)
          sau3AI                                                                                         dpnI(dam+)
          mboI/ndeII(dam-)                                                                               dpnII(dam-)
          dpnI(dam+)                                                                                    bstYI/xhoII
          dpnII(dam-)                                                                           sau3AI  alwI(dam-)
          alwI(dam-)          nlaIII              maeII                                         mboI/ndeII(dam-)
         bstYI/xhoII         rcaI             tru9I                                             dpnI(dam+) mboII(dam-)
       mboII(dam-)           bspHI            mseI                   ddeI hgaI                  dpnII(dam-)
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                       thaI                                                              sau3AI
       sau3AI          fnuDII/mvnI                                                       mboI/ndeII(dam-)
       mboI/ndeII(dam-)                                                                  dpnI(dam+)
       dpnI(dam+)      bstUI                                                             dpnII(dam-)
       dpnII(dam-)     bsh1236I                                                         alwI(dam-)
       alwI(dam-)     hinPI       fnu4HI                             aciI             mspI
      bstYI/xhoII     hhaI/cfoI   bbvI                       aciI   nspBII            hpaII      aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

                bsrI            hinPI                     rmaI                haeIII/palI
            maeIII   eco57I     hhaI/cfoI                 maeI      bslI     haeI                  scfI     aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                           scrFI
                                                                           nciI
                                            fnu4HI                         mspI                     mspI
                                   alwNI    bbvI                           hpaII                    hpaII
                                  bsrI   fnu4HI                            dsaV     pleI              bsaWI      hinPI
          mnlI                maeIII     bbvI    bsrI                      cauII    hinfI          maeIII        hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                                   hgiAI/aspHI
          mcrI                     bsp1286
         aciI                      bsiHKAI
       nspBII                      bmyI                                                                          hinPI
      fnu4HI                       apaLI/snoI                                                                    hhaI/cfoI
      bbvI                         alw44I/snoI    aluI                        ddeI      scfI                   haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

```
                                                                                               scrFI
                                                                                               mvaI         scrFI
                                                                                               ecoRII mvaI
                                             mspI                                              dsaV   ecoRII
                                             hpaII                                             bstNI        dsaV
                                             bslI    fnu4HI                  hinPI  mnlI       bsaJI        bstNI
                                aciI        bsaWI    aciI                    hhaI/cfoI   aluI  apyI[dcm+]   apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                fnu4HI
                                                                                                                aciI
                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                     taqI                            nlaIV                     bstUI
                                    mnlI  drdI    hgaI           sfaNI            aciI                         bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                      tfiI
      haeIII/palI nlaIV haeI        haeI           aflIII                     hinfI                   aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                                       thaI
                                                                                                                       fnuDII/mvnI
                                                                                                                       bstUI
                                                                                                                       bsh1236I
                                                                                                                      hinPI
                                                                                                                      hhaI/cfoI
                                                                                                                     thaI
                                                                                       hinPI                         fnuDII/mvnI
                         fnu4HI           fnu4HI                                   sapI hhaI/cfoI                    bstUI
                         bbvI             bbvI  pleI                               mboII                             bsh1236I
               bsrBI  aciI               hinPI  hinfI                              earI/ksp632I           mnlI       bslI
     aluI      aciI  fnu4HI     mcrI     hhaI/cfoI          mnlI    aciI   haeII                    aciI             aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                 tru9I   aluI
      haeIII/palI       pvuII                                                    tru9I                            nlaIV
     eaeI tfiI   mseI   nspBII                                         hinPI     mseI       maeIII                hgiCI
     cfrI hinfI aseI/asnI/vspI           bsrI     aciI                 hhaI/cfoI aseI/asnI/vspI mnlI              banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

      scrFI
      mvaI
      ecoRII
      dsaV
      bstNI
      apyI(dcm+)           mspI                    aciI                                                    xmnI
     bsaJI                 hpaII                  bsrBI                          aluI       nlaIII         asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

       tru9I
       mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCi/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21
Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21
Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21
Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21
A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21
A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21
A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21
A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

-/--

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document -----	1-4

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9405784	17-03-94	AU-B-	4839493	29-03-94
EP-A-0215548	25-03-87	US-A-	4968626	06-11-90
		DE-A-	3688900	23-09-93
		DE-T-	3688900	09-12-93
		JP-A-	62111690	22-05-87
		US-A-	4959318	25-09-90
		US-A-	5073609	17-12-91
		US-A-	5302529	12-04-94
WO-A-9217566	15-10-92	AU-B-	660671	06-07-95
		AU-A-	1580692	02-11-92
		JP-T-	6506356	21-07-94
WO-A-9012025	18-10-90	US-A-	5043270	27-08-91
EP-A-0260148	16-03-88	AU-B-	613316	01-08-91
		AU-B-	7831787	19-05-88
		DE-A-	3730599	07-07-88
		FR-A-	2603899	18-03-88
		GB-A,B	2197321	18-05-88
		US-A-	5024939	18-06-91
		JP-A-	63152986	25-06-88
EP-A-0160457	06-11-85	AU-B-	601358	13-09-90
		AU-B-	4134585	24-10-85
		AU-B-	5295890	30-08-90
		EP-A-	0385558	05-09-90
		HK-A-	8395	27-01-95
		JP-A-	60243023	03-12-85
		JP-A-	6040942	15-02-94
		NO-B-	174934	25-04-94
		SG-A-	3994	10-06-94
		US-A-	4965199	23-10-90